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FURTHER STUDIES ON THE PARASITISM OF *TAPHRINA DEFORMANS*[1]

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On the Niagara Peninsula, Ontario, peach leaf curl is confined almost exclusively to leaves which develop in the early spring. The absence of leaf curl throughout the summer is interesting inasmuch as new leaves are produced throughout the growing season, and the causal organism, *Taphrina deformans* (Fcl.) Tul., is almost certainly present on peach twigs throughout the year as part of the normal saprophytic micro-flora (*1*). The need therefore of a careful study of the influence of environmental factors on the development of this disease is apparent. The present paper presents the initial results of a study of the effect of temperature and moisture upon the process of penetration and upon the subsequent development of the disease.

The Conditions Requisite for Penetration

A preliminary study of the conditions requisite for penetration was made both with detached shoots collected in the summer and with potted plants forced in the greenhouse during the latter part of the winter. Inoculations were made either by spraying the developing leaves with a spore suspension using an atomizer or by painting the suspension on them with a fine brush. The shoots or plants, as the case might be, were then placed in moist chambers for the duration of the particular experiment. In order to determine whether or not penetration had occurred, the leaves were boiled in lacto-phenol cotton blue until they were cleared and then examined microscopically for infection centres in which the stained mycelium stands out quite conspicuously from the cleared leaf tissue.

The results of a number of such experiments have shown that penetration can occur at temperatures ranging from 50° to 70° F. Within this range mycelium could be found in the leaves 48 hours after the plants were inoculated. Between 45° and 50° F. some penetration did occur but this was distinctly limited. The optimum temperature for penetration probably lies somewhere between 55° and 65° F. but, for reasons which will be apparent later, it is difficult to determine this with any degree of certainty.

The Relation Between Environment and Subsequent Development of Leaf Curl

In this series of experiments potted peach trees which had been inoculated under similar conditions were made to complete the development of their foliage in different environments. As a preliminary experiment eight

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FIGURE 1—Four of a group of eleven plants which after the buds had broken were inoculated and incubated for 48 hours at 50° to 65° F. and then transferred for 18 days to a constant temperature chamber at 70° F. and 80% relative humidity.

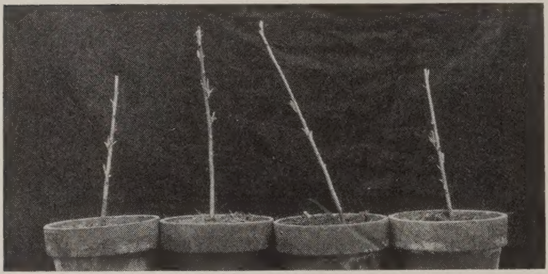

FIGURE 2—As in Figure 1 except that these plants were held at 50° instead of 70° F.

FIGURE 3—Plants of the same lot as in Figure 2 six days later having been held for that time at 60 ° F.

small trees, whose buds were broken so that the first leaves were exposed, were sprayed with a spore suspension of sprout conidia and placed in the inoculating chamber for six days. The temperature of the chamber was held at 45° to 50° F. for the first four days but, as so little penetration occurred during this time, it was decided to raise the temperature to 55° to 60° F. and allow the plants to remain in the chamber for two days longer. When the plants were removed from the chamber they were divided into two lots of four each. One lot was placed in a warm greenhouse (65° to 75° F.) and the other in the coolest house available (45° to 60° F.). At the end of three weeks from the time of their removal from the moist chamber the buds of those plants in the warmer house had developed shoots ranging in length from 6 to 11 inches, but of these only one showed any leaf curl. On the other hand, the buds of the plants in the cooler house had developed more slowly and none of the shoots exceeded six inches in length. On every one of these plants a number of curled leaves developed.

In the next series of experiments temperature and moisture were very closely controlled. The plants were inoculated as before and held in the moist chamber for 48 hours, the temperature varying between a maximum of 65° and a minimum of 50° F. during this period. When they were taken from the moist chamber they were grouped in matched pairs and then divided into two lots each of which contained one of a pair of corresponding individuals. In this way a satisfactory distribution of experimental material was assured. The plants were then placed in two constant temperature chambers, one of which was kept at 70° and the other at 50° F., the relative humidity in each case being 80%. Those in the chamber at 70° F. received 8 hours illumination per day from eight 500-watt tungsten bulbs whereas those at 50° F. received 4 hours per day from a similar source. The effect of this difference in treatment soon manifested itself in the respective rates at which the plants in the two chambers grew. The buds of those in the chamber at 70° F. developed rapidly and within nineteen days produced the foliage shown in Figure 1. On the other hand the plants in the chamber at 50° F. made so little development in the corresponding time (Figure 2) that it was decided to raise the temperature to 60° F. and to increase the illumination to 8 hours per day.

Leaf curl could first be detected on the plants at 70° F. eight days after they were placed in the chamber, but on eleven plants in this chamber only seven curled leaves were developed which were typical and produced asci. A few other leaves on these plants did show indications of being infected during the early stages of their development but they seemed to outgrow the infections as they did not become curled and no asci were produced. On the corresponding eleven plants which were in the 50° F. chamber leaf curl could not be detected until fifteen days after they were placed in the chamber. When the shoots finally did develop every plant showed a heavy incidence of curl and seventeen of the forty-eight shoots were so severely diseased that they developed practically no healthy leaves (Figure 3). The infections on these plants all appeared to be normal ones and in every case asci were produced on the diseased areas when the leaves matured.

DISCUSSION AND CONCLUSIONS

Since penetration of young peach leaves by *T. deformans* occurs readily between 50° and 70° F. and these temperatures are well within our normal summer range, the disappearance of the disease during the summer cannot be due to an inability of the fungus to enter the leaf, but must rather be attributed to factors operating after penetration has taken place. This is further suggested by the fact that the conditions under which the plants grow after penetration has occurred materially influence the amount of leaf curl which develops, those grown at high temperatures developing little or none whereas those grown at lower temperatures become heavily diseased. It seems likely therefor that during the summer new leaves develop so rapidly that their period of susceptibility is passed through before the fungus can establish adequate parasitic relationship within the leaf and that consequently such infections are unable to develop beyond the initial stages, the fungus dying as the leaf matures. That such a susceptible period exists and that later the tissues become resistant to any further spread of the fungus is suggested by the well defined boundary which always exists between healthy and diseased areas on the mature leaf. This resistance seems to be correlated with the degree of maturity of the tissue inasmuch as the tip which matures earliest (*2*) is the most resistant part of the leaf. A histological study of abortive infections on rapidly-growing leaves is being made with the hope of finding out something further of the host-parasite relationships.

These results indicate further that the prevalence of leaf curl following cold wet springs is to be accounted for in large part by the slow growth rate associated with them being optimum for the later stages of the infection process rather than to them offering peculiarly optimum conditions for the initiation of infection.

LITERATURE CITED

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Résumé

Nouvelles études sur le parasitisme du *Taphrina deformans*. R. E. Fitzpatrick, Vineland Station, Ontario.

Il a été démontré par des recherches préliminaires sur des pêchers plantés en pots que les phases initiales de l'infection par *Taphrina deformans* peuvent se produire à une température de 50° F. et jusqu'à 70° F., mais que la gravité de la maladie (l'enroulement des feuilles du pêcher) est grandement influencée par les conditions dans lesquelles les plantes sont cultivées après que l'on sait que la pénétration s'est produite. Aux hautes températures (70° F.), lorsque le développement des feuilles était rapide, les relations parasitaires n'ont pas été maintenues et le champignon a été détruit. Aux températures plus basses (50° F. — 60° F.), lorsque le développement des feuilles était lent, le champignon a pu s'établir plus promptement et il en est résulté une haute fréquence de l'enroulement des feuilles.

SECONDARY TUMOR FORMATION ON HERBACEOUS HOSTS INDUCED BY *PSEUDOMONAS TUMEFACIENS* Sm. and Town.[1]

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INTRODUCTION

A series of 300 two-year old apple trees in the experimental plots at the Iowa State College, which had been inoculated with *Pseudomonas tumefaciens* Sm. and Town. the previous summer, showed the development of conspicuous swellings on several of the trees during the summer of 1929. These swellings, which were, in all probability, secondary tumors, were situated from eight to ten inches above the point of inoculation and were from one-fourth to three-fourths of an inch in diameter, but were not convoluted externally as is a typical crowngall.

It is possible that the swellings found might be other than secondary tumors. However, various facts seem to point to the secondary tumor as the explanation. The soil in which the trees were growing had been steam sterilized for 40 minutes at 120 pounds pressure with an inverted pan before the trees were planted. This fact would reduce the possibility of any natural infection coming from the soil. A close examination of these swellings showed that the bark layer was not broken. Therefore, in all probability they were not caused by callus formation induced by a wound. The tumors were split and examined to determine whether insect irritation could have been the cause. No evidence of insects or insect injury was found.

While it appears that these swellings were secondary tumors, the exact manner of their formation is questionable. In considering the possible methods of the formation of secondary tumors, it does not seem probable that tumor strands, as reported by Smith *et al* (*12*), could have forced themselves through the apple tree tissue to such a distance, and also no evidence of tumors was found between the points of inoculation and the secondary tumors. The explanation as given by Riker (*10*), Robinson and Walkden (*11*) and Levine (*6*) that secondary tumors are caused by elongation of the growing point after inoculation in this region does not apply since no growing point was involved here. Also it does not seem likely that the wound made when inoculating could have exerted such an influence by a release of liquid in the intercellular spaces as reported by Riker (*9*) and Invanoff and Riker (*5*), nor would it be probable that the organisms had migrated through the intercellular spaces to such a distance as zoogloeae as reported by Robinson and Walkden (*11*) and Hill (*3*). The possibility that the crowngall organism might pass through the xylem of the vascular system of a plant and form tumors a considerable distance from the point of entrance suggested itself and was studied. Hill (*3*) and Hamdi (*2*) have suggested that *Ps. tumefaciens* could migrate in the xylem

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of a plant. Hill *et al* (*4*) have found zoogloeae migrating short distances in the vessels, while Riker (*9*) states that the organism may travel in some parts of the vascular bundles if the conditions are favourable.

MATERIALS AND METHODS

Experiments were started at the Iowa State College and continued here to develop the concept of vascular invasion of the host by *Ps. tumefaciens*. Herbaceous plants were chosen for experimentation inasmuch as they are easily grown and react rapidly to the crowngall organism. Four herbaceous plants have been used; namely, Paris daisy (*Chrysanthemum frutescens* L.), tomato (*Lycopersicon esculentum* Mill. var. Dwarf Stone), tobacco (*Nicotiana tobaccum* L.) and *Bryophyllum calycinum* Salisb. The culture of *Pseudomonas tumefaciens* used throughout the experiments was isolated by Dr. J. H. Muncie from a gall on an apple tree in 1926 and designated by him No. 468. The fact that galls were obtained in every case at the point of inoculation would indicate the virulence of the culture.

Throughout the experiments two types of inoculation were employed: the broth-culture method and the needle method. In the broth-culture method tomato plants 10–12 inches, Paris daisy plants 4–6 inches, tobacco plants 10–12 inches, and Bryophyllum plants 8–10 inches high were used. The plants were cut off one half inch above the soil and stood with the lower half inch of the stem in a two-day-old broth culture of *Ps. tumefaciens*. They were then set in the sunlight, to induce transpiration, for four hours. The plants were then taken out and all the leaves except the topmost two removed by cutting the petiole of each at its juncture with the stem. In all cases the knife used for cutting was flamed before each cut. These cuttings were then set in sand which had been steam sterilized at 20 pounds pressure for one hour, and were kept shaded for the first week.

TABLE 1.—EFFECT OF LENGTH OF TRANSPIRING PERIOD ON THE MOVEMENT OF *Ps. tumefaciens* THROUGH THE STEM OF DWARF STONE TOMATO PLANTS

Time	Number of cuttings	Location of galls				
		Base	1st node	2nd node	3rd node	4th node
¼ hr.	40	40	10	34	11	0
½ hr.	40	35	20	35	0	0
1 hr.	40	37	37	14	15	0
2 hrs.	40	40	20	40	18	0
4 hrs.	40	40	36	40	40	26

A preliminary experiment was conducted in order to determine the correct transpiring period for the broth-culture method in which the cut plants were allowed to remain in the broth culture of *Ps. tumefaciens* and in the sunlight for different lengths of time. Table 1 shows the results from this experiment. It is evident that the four-hour period resulted in the larger number of secondary tumors being formed. Periods longer than four hours were tried but did not show any perceptible increase in the number of secondary tumors.

The needle method is much simpler and is the usual method of inoculating hosts with the crowngall organism. A small amount of a three-day-old agar culture of the organism is taken on the tip of a spear-point needle. This mass of bacteria is put on the host at the desired place and

introduced into the tissue by a single stab through the mass or by several pricks into the tissue through the mass of bacteria. The operation wounds the tissue of the host and introduces the organism. Plants that have been inoculated are then placed in a moist chamber for three days, after which they are returned to the greenhouse bench.

Many isolations were made throughout the course of these experiments. All isolations were made with the bile crystal violet medium following Patel's (*8*) technique for the isolation of *Ps. tumefaciens.* Better results were obtained in plating when a sterile salt solution of 5 gms. sodium chloride and 1 gm. magnesium sulfate per liter of distilled water was used in place of sterile distilled water. Colonies were selected from the poured plates and transferred to potato dextrose agar slants. These cultures were then inoculated into tomato to prove the presence of *Ps. tumefaciens.*

Material for histological study was killed in the acetic acid-formalin-alcohol solution and dehydrated with acetone. Safranin with a counterstain of fast green S.F. was used for staining sections.

INOCULATION STUDIES

Evidence as to the movement of the organism through the host stem was sought in two ways. In the first case efforts were made to isolate the organism from the stem at various distances from the point of inoculation, while in other experiments the formation of secondary tumors was considered to be possible evidence of movement of the organism.

Results of Isolations Following Inoculation

Distance of Movement of Ps. Tumefaciens

Three series of 30 plants each of tomato, Paris daisy and Bryophyllum plants were inoculated by the broth-culture method. The isolations from the tomato cuttings immediately after treatment showed 100% positive for *Ps. tumefaciens* at two and four inches from the base of the cutting, while isolations at a distance of six inches were 8% positive. In the case of the Bryophyllum cuttings 100% of the isolations showed *Ps. tumefaciens* at a distance of two inches, and at three inches 25% of the isolations were positive. With Paris daisy cuttings all isolations at one inch above the base were positive, with only 3% of the trials at two inches resulting in *Ps. tumefaciens* being obtained.

The movement of the crowngall organism down the stem and the possible influence of transpiration stream were observed on tomato plants. Two series of 25 plants each were inoculated by attaching tubes of a two-day-old broth culture at the tip of the stem, while two other series were inoculated at two of the lateral leaf petioles of each plant for four hours. Isolations were made at one inch and two inches below the cut tip of the plant. Positive results were obtained one inch below the point of introduction of the organism and negative results at the second place of isolation. When the organism was introduced through the petioles, which were one and one half inches long, isolations were made at the point of union of the petiole and stem, one and two inches below, and one and two inches above the juncture. Positive isolations were obtained at the juncture of

the petiole and stem and also one inch above. The other points of isolation, however, gave negative results. These results would indicate that *Ps. tumefaciens* moves more rapidly up the stem and is influenced by the transpiration stream contrary to Hill (*3*), who reports that the migration was independent of the flow of sap in the vessels.

To determine whether *Ps. tumefaciens* travelled in the xylem of the host plant, 50 Bryophyllum plants were ringed to remove the phloem and cortex. The ringed portion was three-fourths of an inch wide. The cuttings were then inoculated by the broth culture method, and after treatment isolations were made from the stem one inch above the ringed portion. In all the isolations *Ps. tumefaciens* was recovered in practically pure culture. The results of this experiment would indicate that *Ps. tumefaciens* travels through the vessels of the xylem, inasmuch as the distance travelled was over 500 mm., while Hill (*3*) and Ivanoff and Riker (*5*) report the organism to travel as zoogloeae through the intercellular spaces of the cortex and pith to a distance of 3 to 5 mm. in four hours. The rate of movement of zoogloeae in the vessels as reported by Hill *et al* (*4*) is also much slower.

Longevity of Ps. tumefaciens within host tissue

The longevity of *Ps. tumefaciens* within the host tissue without producing pathic events is questionable. One set of 100 tomato plants were inoculated by the broth-culture method and isolations were made from six cuttings each week at points three, five and seven inches above the base of the cutting. The experiment was repeated three months later. Isolations continued for 10 weeks in each case, at which time the cuttings were starting to die from lack of nutrients as they were being grown in steam sterilized sand.

These isolations showed that after 10 weeks *Ps. tumefaciens* could still be isolated from an apparently normal internode of the tomato cutting. Isolations made during both 10-week periods from the point three inches above the base of the cutting showed 92% as positive and from the point five inches above 60%, while the isolations from the 7-inch distance were all negative. The growth of *Ps. tumefaciens* on the agar plates was of interest. In the case of isolations from the 3-inch lot the colonies appeared from two to three days after the isolation was made. The plates made from the 5-inch lot never showed colonies until 4 or 5 days after the isolation. The cause for this difference in the time of appearance of the colonies on the agar plates might be that the organisms farther up the stem had become attenuated. However, inoculation of the transferred colonies into tomato plants to establish the presence of *Ps. tumefaciens* did not show any difference in the virulence of any of the cultures. It was also noticed that the isolations for the 8th, 9th and 10th weeks were a day later in developing colonies of the organism than was normal.

The results of the various experiments to determine the distance of movement of *Ps. tumefaciens* through the host show that the organism moves rapidly in the xylem to a distance of at least six inches in tomato, three inches in Bryophyllum, and two inches in the Paris daisy. The movement of the organism down the stem through the xylem reaches a distance of one inch in four hours, while the transpiration stream aids in the movement of the organism upward. Check inoculations were made in every experiment, using sterile broth. All isolations in the check treat-

ments were negative for *Ps. tumefaciens*, while over 95% of the plates remained sterile.

Formation of Secondary Tumors Following Inoculation

A further test of the distance of movement of *Ps. tumefaciens* through the host plant is the formation of secondary tumors. Although the previous experiments showed that *Ps. tumefaciens* could move through the host plant for a certain distance, they did not indicate whether the organism could induce secondary tumors at these various distances from the point of entrance.

Inoculation by the broth-culture method

At various times during a period of two years, six series of 100 tomato plants each have been inoculated by the broth-culture method and final notes taken 35 days after the start of the experiment. In most cases it was observed that tumors had started to form on various cut petioles within 12 days after inoculation. Table 2 shows a summary of the results obtained from the six experiments.

TABLE 2.—DISTRIBUTION OF SECONDARY TUMORS ON TOMATO CUTTINGS INOCULATED BY THE BROTH-CULTURE METHOD WITH *Ps. tumefaciens*

Number of cuttings	Position of tumor	Cuttings with tumors	
		Number	Percentage
600	Base of cutting	600	100.0
600	1st node	570	95.0
600	2nd node	510	85.0
600	3rd node	474	79.0
600	4th node	390	65.0
600	5th node	306	51.0
436	6th node	151	34.6
321	7th node	93	29.0
164	8th node	39	23.7
45	9th node	8	17.7
600	Extruding on internode. Lower half of stem.	592	98.6
600	Extruding on internode. Upper half of stem.	387	64.5
100	Check	0	0

These results show that secondary tumors are found as far as the 9th node from the base, which is about 16 inches from the place of entrance. It is noticed that the number of cuttings is less beginning with the 6th node. This has happened because not all of the cuttings used were of the same height. It is also observed that the percentage of cuttings showing secondary tumors at the cut petioles is less as the distance from the point of entrance increases. Table 2 further shows that secondary tumors were formed on the uninjured internodes of the inoculated cuttings. As many as 12 of these tumors have been observed on one plant. The distribution of these secondary tumors found extruding through normal tissue is approximately the same as the tumors formed at the cut petioles.

Two series of 100 tobacco plants each were treated similarly to the tomato plants. The results of these experiments show that the percentage

of cuttings with secondary tumors at the cut petiole varied from 92% at the 1st node to 14.5% at the 7th node, a distance of 12 inches. All of the cuttings produced tumors at the base, while 18% of the cuttings showed secondary tumors extruding on the internode. These results are in accord with those obtained with the tomato plants.

Similar experiments to those carried on with tomato and tobacco plants were conducted with Bryophyllum and Paris daisy plants. In these experiments galls were formed at the base of all of the cuttings, but no secondary tumors were observed. However, the isolation experiments showed that *Ps. tumefaciens* had moved a distance of three inches in the Bryophyllum plants and two inches in the Paris daisy plants.

Effect of Wounding on the Formation of Secondary Tumors

Three experiments were conducted with tomato and tobacco plants inoculated by the broth-culture method, in which wounds were made with a sterile knife on the fourth internode (about five inches) from the base of the cutting. Tomato cuttings wounded within one week after inoculation showed the formation of tumors at 94% of the wounds within four weeks, while wounding three weeks after inoculation showed tumors at 45% of the wounds in the same period of time. In the case of the tobacco plants the percentage of tumors formed at the wounds varied from 57% on the cuttings wounded immediately after inoculation to 5% on the cuttings wounded 35 days after inoculation. In the above experiments transverse wounds were made to a depth of about one third of the diameter of the stem.

The wounds made in the above experiments would, of course, wound both the vascular bundle and also the surrounding medullary rays. In order to determine if wounding in the medullary ray would induce tumor formation two series of 100 young tomato plants each were inoculated by the broth culture method. A study of the vascular skeleton of the tomato plant showed the presence of a ring of vascular bundles separated by medullary rays. Half of each series was wounded with a single needle stab through the medullary ray while the other half of the series was wounded through the vascular bundle. Each cutting was wounded at five places from three to eight inches from the base. In Table 3 is shown the com-

TABLE 3.—EFFECT OF PLACE OF WOUNDING INOCULATED TOMATO CUTTINGS ON THE FORMATION OF SECONDARY TUMORS BY *Ps. tumefaciens*

Height from base of cutting	Wounded in vascular bundle				Wounded in medullary ray			
	Number of cuttings	Number with tumors	Number without tumors		Number of cuttings	Number with tumors		Number without tumors
			Stab in vascular bundle	Stab in medullary ray		Stab in vascular bundle	Stab in medullary ray	
3 inches	100*	67	14	19	100	7	0	93
4 inches	100	69	12	19	100	8	0	92
5½ inches	100	74	12	14	100	8	0	92
6½ inches	100	65	18	17	100	4	0	96
8 inches	100	58	27	15	100	3	0	97

*Cuttings wounded one week after inoculation and final notes four weeks later.

bined results of the two experiments. The percentage of wounds in the vascular bundle that resulted in the formation of tumors varied from 82.7% at three inches from the base of the cutting to 68.2% at a distance of eight inches. Some cuttings showed tumors at every wound and only six cuttings showed no tumors at any of the wounds. The wounds made through the medullary ray did not result in the formation of secondary tumors. These results indicate that if *Ps. tumefaciens* is in the vascular system of a plant, wounds which injure this tissue will probably result in the formation of tumors. Also these experiments again show that the percentage of tumors becomes smaller as the distance from the point of entrance increases.

In another experiment 100 tomato cuttings that had been inoculated by the broth culture method were each cut off six inches above the base with a sterile knife nine weeks after inoculation. These upper portions of the cuttings were then set in steam-sterilized sand. Examination of these after three weeks showed that 76% of them had produced galls at the base. These results would indicate that it would not be advisable to take cuttings from a plant severely infected by *Ps. tumefaciens*.

Inoculation by the needle method

The development of secondary tumors following the inoculation of *Ps. tumefaciens* by the needle method has been studied with respect to the four hosts used in previous experiments.

Two series of 100 tomato cuttings each were inoculated at the base with *Ps. tumefaciens* by the needle method and then set in steam-sterilized sand. The cuttings in each case were examined after 30 days. All of the cuttings showed galls at the base, while the first node showed 17%. Tumors were not found at any of the other nodes, and no tumors were observed extruding on the internodes. In comparing these results with those from the broth-culture method of inoculation, it would seem that the formation of secondary tumors at a distance from the primary tumor was determined by the number of organisms gaining entrance to the vessels of the xylem.

In another experiment tobacco plants were inoculated by the needle method in the growing point and at different distances up to three inches below. Ninety-four per cent of the plants inoculated in the growing point showed the presence of secondary tumors. This high percentage of secondary tumors was expected, however, as Riker (*10*) and Robinson and Walkden (*11*) have shown this to be a way in which secondary tumors may be formed. Inoculation one-half inch below the growing point resulted in 60% of the plants showing secondary tumors; one inch below, 50%; two inches below, 40%; and three inches below, 80%. These secondary tumors were from one-fourth to two inches from the gall at the point of inoculation. Of the secondary tumors formed, 74% were above the point of inoculation and 26% were below. The former were distributed at greater distances from the point of inoculation. This fact also supports the contention that the transpiration stream has an influence on the movement of *Ps. tumefaciens* in the vessels of the host. One other fact indicated by inoculations in this experiment is that wounding of the stem with many needle stabs in inoculation is more conducive to secondary tumor formation than a single stab of the needle, although severe wounding of the

stem did not perceptibly increase the number of secondary tumors produced. This fact is explained, however, by the larger number of vessels that would be broken in the wounding of an area of the stem one-fourth inch square.

Paris daisy plants were inoculated by the needle method in the growing point, one-fourth inch and two inches below the growing point. Thirty per cent of the plants inoculated in the growing point developed secondary tumors. The inoculation one-fourth inch below the growing point resulted in the production of secondary tumors on 34% of the plants, while in the inoculation two inches below, 10% of the plants showed secondary tumors. In all cases galls were formed at the point of inoculation.

Inoculations were made on Bryophyllum plants in the growing point and one-half, one, and two inches below the bud. No definite secondary tumors were formed, although in 52% of the plants inoculated in the growing point, the resulting galls extended over two and in some cases three internodes. In all other plants, galls were formed at the point of inoculation.

In comparing these two methods of inoculation for secondary tumor formation, the number of tumors formed and the distance travelled by *Ps. tumefaciens* is much greater in the case of the broth-culture method. However, this difference should be expected as the broth culture method introduces a great number of organisms into the vascular tissue, while in the needle method only comparatively few would be introduced and then only if the vascular tissue was broken by the needle.

HISTOLOGICAL STUDIES

The investigations so far have been concerned with the reactions of the host when inoculated with *Ps. tumefaciens* in various ways. This information, however, does not explain why some hosts show a larger number of secondary tumors than others, what tissues are definitely the place of migration of the organism, or how the secondary tumors originate. Histological studies of the hosts and of secondary tumors have been made in an effort to explain these points.

Normal Anatomy of the Four Hosts

In cross section, the young stems of the four hosts show a peripheral ring of vascular bundles separated by medullary rays. As the stems increase in age interfascicular cambium forms so that a cross section shows a complete ring of vascular tissue. Examination of the xylem shows that this tissue is composed chiefly of xylem parenchyma with a relatively small number of vessels occurring in groups.

The relation of the leaf trace to the vascular skeleton of the stem among these plants is of two general types. In the tobacco and tomato plants the leaf traces are branches of the main vascular bundles running through the stem similar to the vascular system of the potato plant as reported by Artschwager (*1*). In the Paris daisy and Bryophyllum plants it appears that the leaf traces are strands of vascular tissue extending from the crown of the plant, so that the xylem in one petiole has no connection with the xylem in any other portion of the plant.

Another point of interest is the size of the vessels in each host. The vessels in tomato and tobacco vascular tissue have approximately twice the diameter of the vessels found in the Paris daisy and Bryopyhllum. Also it was found that the vessels in the upper one-third of the stems of tomato and tobacco were about one-half the diameter of most of the vessels found in the older portions. In the case of Paris daisy and Bryophyllum the vessels in the upper portion of the stem were about two-thirds of the diameter of the vessels in the older portion of the stem. Also it was found that in the xylem region of the stems of tomato and tobacco plants there were twice as many vessels per unit area as were found in the Paris daisy and Bryophyllum plants.

This difference in vascular anatomy between tomato and tobacco plants and Paris daisy and Bryophyllum plants and the difference in the size and number of the vessels probably explain the difference in the number of secondary tumors formed on the plants in each group when inoculated by the broth culture method. The smaller number of secondary tumors formed on the upper one-third of the inoculated stems might be explained by the fact that the vessels are smaller in this region and the number of organisms that could move here would be less.

Presence of Ps. tumefaciens in the vessels

The results obtained from the various inoculation experiments indicate that *Ps. tumefaciens* moves in the vessels of the host. Observation of the organism in the vessels would be more definite proof that such is the case. Free-hand selections of tomato stems inoculated by the broth-culture method with a suspension of the stained organism did not show the organisms, although the walls of the vessels were stained. Sections were also made from tomato stems inoculated by the broth-culture method and then stained. In some instances *Ps. tumefaciens* was thought to be found in the vessels. The staining reaction of the organism is practically the same as the host tissue, so a further experiment was conducted in which a stained suspension of *Mycobacterium berolinensis* was introduced. This organism is approximately the same size as *Ps. tumefaciens* and is acid fast. Free-hand sections of tomato stems inoculated with *M. berolinensis* showed the organism to be present in the vessels up to six inches above the point of entrance.

Origin of Secondary Tumors

Experiments have shown that when a plant which had been previously inoculated by the broth-culture method is wounded so as to break the vessels at a point above the place of inoculation, secondary tumors frequently form at this point. It appears that the breaking of the vessels allows the organisms to accumulate with a consequent action of the organism in inducing a tumor. Microscopical examination of sections of tomato stem inoculated by the broth-culture method has shown internal tumors in the protoxylem region. Plate I A shows a small internal tumor. These internal tumors are usually situated some distance from the surface of the stem and it is doubtful if they would ever reach the surface. Further, these internal tumors appear to originate from the vessels of the protoxylem that have been broken by elongation of the stem.

The formation of secondary tumors that appear on the surface of the uninjured internode of the stem is more difficult to explain. Plate I

B shows a longitudinal section of a tomato stem showing a secondary tumor starting to form. A small tumor is forming adjacent to the vessels, and above and below other vessels have formed. Also it appears that the organism has also influenced the tissue just outside of the longitudinal fiber and also the tissue at the surface of the stem. A cross section of a tomato stem showing a young tumour apparently originating from the xylem and forcing its way outward is shown in Plate II A. Tracheids can be seen connecting this growth with the vessels of the xylem.

An older secondary tumor shows more clearly the method of development. Plate II B is a diagrammatic drawing made with the aid of a camera lucida. Here is shown a definite strand of tracheids originating from the xylem and then being distorted and broken up in typical crowngall fashion. Structures resembling adventitious roots were found in connection with many of the secondary tumors examined. It is probable that an accumulation of *Ps. tumefaciens* in the vessels acts in stimulating the formation of adventitious roots, through the vessels of which the organisms pass. After penetrating the phloem and the fiber ring into the cortex, the adventitious growth, under stimulus of the organism, is finally distorted and formed into gall tissue as it breaks through the epidermis, thus forming a tumor on the surface of the stem. Recently Levine (*7*) has reported the presence of young adventitious roots in crowngall on Opuntia. However, he reports that these young rootlets become abortive and necrotised.

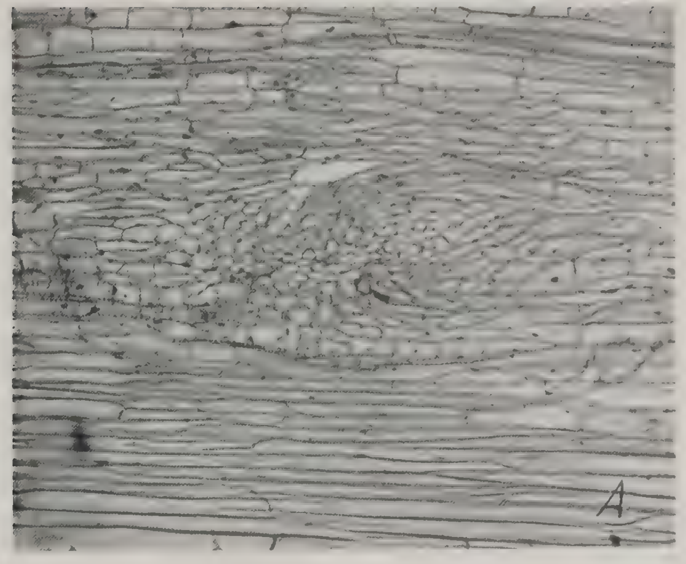
DISCUSSION

The passage of phytopathogenic bacteria through the vessels of the xylem of the host is not new. It is well known that in many bacterial diseases the organisms move through the vessels. The probability that *Ps. tumefaciens* could travel through the vessels of the host has been suggested by Riker (*9*), Hill (*3*), Hill *et al* (*4*) and Hamdi (*2*). Definite proof has been obtained that such is the case. The distance of movement has been shown, by isolation of the organism, to be as far as six inches in four hours and, from the location of secondary tumors, apparently as far as 16 inches. This is a much greater distance than that previously reported. Hill (*3*) and Ivanoff and Riker (*5*) showed that the organism could move as far as four millimeters in four hours, but after that time the movement became less, while Hill *et al* (*4*) report the movement of zoogloeae in the vessels to a distance of 2.3 millimeters in 15 minutes. It appears that *Ps. tumefaciens* can move through the vascular system of the host but is limited by the anatomy of the xylem and the size of the vessels.

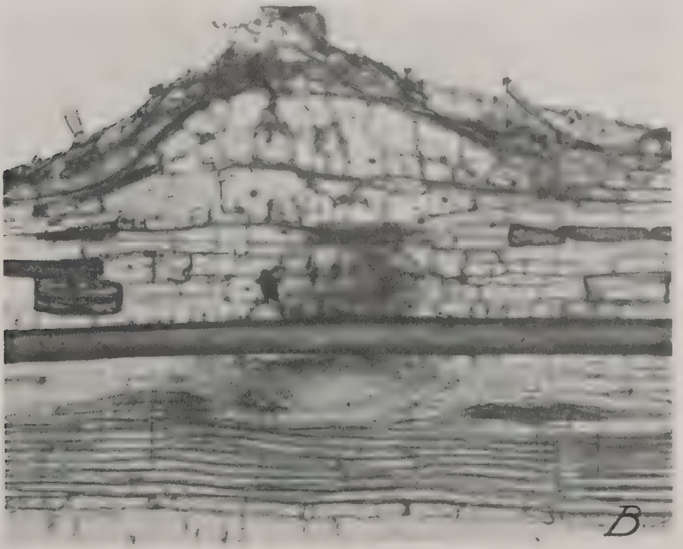
The movement of *Ps. tumefaciens* through the vessels is aided by the transpiration stream. This is indicated by several facts. In the broth-culture method of inoculation more secondary tumors were formed, and also at a greater distance from the point of entrance, as the length of time the plants were kept in the sunlight was increased. When inoculated into the tip of tomato plants the organism showed only a very short movement down the stem. When the organism was introduced into a petiole it was recovered above the node and not below. Further evidence was obtained from the inoculation of the tobacco plants by the needle method. In this a much larger percentage of tumors formed above the point of inoculation and had a wider distribution along the stem than those formed below.

DESCRIPTION OF PLATES

PLATE I

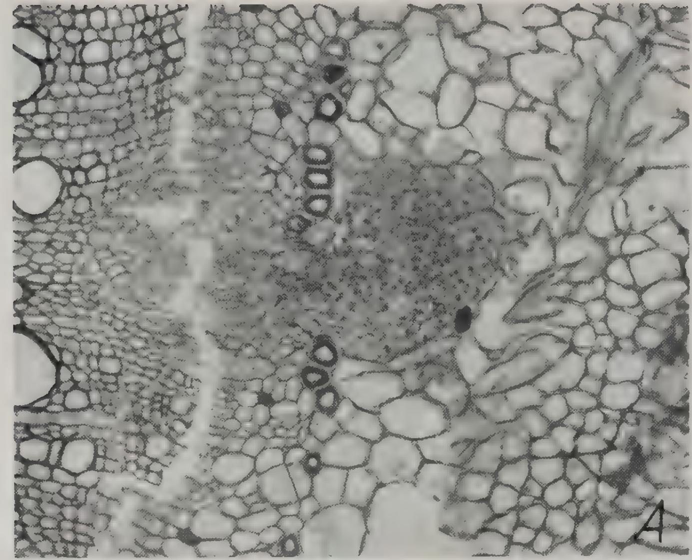


A.—Longitudinal section of a tomato stem 35 days after inoculation showing a small internal secondary tumor in the protoxylem region. × 128.



B—Longitudinal section of a tomato stem showing a young secondary tumor forming adjacent to the xylem and influencing the cortex and epidermis. × 64.

PLATE II



A.—Cross section of a tomato stem showing a young secondary tumor originating from the xylem and forcing its way outward. × 128.

B—Camera lucida drawing of a cross section of a tomato stem showing an extruding secondary tumor that originated from the xylem and has formed on the surface of the stem. × 110.

These results, however, do not support Hill's (*3*) statement that "the bacteria were found in the vessels both above and below the puncture, indicating that the migration was independent of the flow of sap in the vessels."

The question of the longevity of the crowngall organism in host tissue without producing pathic events is of interest. Isolations showed that *Ps. tumefaciens* could be obtained from apparently normal internodes of a tomato stem for 10 weeks following inoculation. It is probable that the organism could live longer than this period inasmuch as 75% of the isolations made the tenth week were positive. However, the organism appeared to be losing its vitality as it took one to two days longer for the colonies to appear after isolations made the eighth, ninth and tenth weeks. It is possible that in making the isolations, an internal tumor was included in the tissue taken for isolation. If such was not the case, apparently *Ps. tumefaciens* had accumulated and remained inactive in some of the vessels. The longevity of the organism within the host tissue is also important with respect to the formation of tumors on cuttings. Cuttings made from tomato cuttings that had been inoculated nine weeks previously showed that 76% of them developed crowngall in three weeks. It appears, from this information, that it would probably not be advisable to take cuttings, or use other methods of vegetative reproduction, from plants that were severely infected with *Ps. tumefaciens*.

The results show that there are three different ways in which secondary tumors may develop as a result of vascular infection. In the first case, if wounds occur from the exterior which break the vessels, the organisms are freed and induce gall formation. In this case a wound is present and the formation of the tumor is identical with that of galls formed by the usual needle inoculation into the cortex of the stem. Another place of secondary gall formation is the protoxylem region. Here internal secondary tumors are found in connection with the broken vessels of the protoxylem. Smith *et al* (*12*) report the presence of tumor strands in the protoxylem region. However, many of the photomicrographs in their article appear to suggest tumor formation from vascular infection. In several of the plates in Smith *et al* (*12*) ruptured vessels can be observed in connection with the tumor. It is possible that many of the so-called tumor strands reported by Smith *et al* (*12*) to be present in the protoxylem region were internal secondary tumors formed at the places where the vessels had been ruptured due to elongation of the stem. In the third instance secondary tumors are found extruding on the surface of the internodes of inoculated stems. These secondary tumors are not present until about three weeks after inoculation. They increase in number as the time from the date of inoculation increases. It appears that the organisms accumulate in a few of the vessels due to mechanical plugging or at imperfections in the vessels. This group of organisms then seems to induce the formation of a rootlet which forces its way through the cortex and as it appears on the surface of the stem it is disorganized into a tumor. *Ps. tumefaciens* has been isolated from these secondary tumors and it is probable that the organism passes through the vascular tissue of these rootlets. The size of these extruding secondary tumors varies from one-sixteenth to one-fourth of an inch in diameter depending on their age. Young rootlets, or

the remains of the vascular system of such are found in connection with a majority of the extruding secondary tumors examined. As many as four young rootlets about one millimeter long have been observed in the formation of this kind of tumor. When such is the case the tumors are larger and appear to be made up of smaller centers of gall tissue. Several such galled areas have been found to be one-half inch long and one-fourth inch wide.

From the information available with regard to secondary tumor formation there appears to be three ways in which this kind of tumor may form. In the first place, inoculation of plants in the growing point result in the formation of secondary tumors due to the elongation of the inoculated tissue as has been shown by Riker (*10*), Robinson and Walkden (*11*) and Levine (*6*). Secondly, when inoculations are made in the cortex of the stem the organisms migrate short distances from the point of inoculation as zoogloeae, which has been reported by Robinson and Walkden (*11*), Hill (*3*) and Ivanoff and Riker (*5*). Then, lastly, when *Ps. tumefaciens* gains entrance to the vessels, it may move considerable distances and induce secondary tumors, as reported in this work.

It must be remembered, however, that of all this experiment work (Smith *et al* (*12*), Riker (*9, 10*), Robinson and Walkden (*11*), Levine (*4*), Hill (*3*), Ivanoff and Riker (*5*) and herein reported) has probably been done with organisms in excess over those present in nature. However, wounds which penetrate the xylem offer the possibility even in nature of helping to induce secondary tumors on those plants infected by *Ps. tumefaciens*. Also should the organism gain entrance to the vessels, extruding secondary tumors could be formed. On the other hand, it is very seldom that secondary tumors are observed under natural conditions. The one exception might be the cane gall of raspberry.

SUMMARY

The movement of *Ps. tumefaciens* through the vessels of the xylem of the host is demonstrated. This movement is influenced by the transpiration stream, the number and size of the vessels and the vascular anatomy of the stem of the host.

Pseudomonas tumefaciens has existed in the tissue of apparently normal internodes of tomato plants without producing visible pathic events for ten weeks.

Secondary tumors arising from vascular infection by *Ps. tumefaciens* are of three kinds: (1) external tumors, which arise from wounds from the exterior, which break the vessels; (2) internal tumors at breaks in the vessels of the protoxylem; (3) extruding tumors, which originate from the xylem as adventitious growths.

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Résumé

Formation de tumeurs secondaires sur des hôtes herbacés provoquée par *Pseudomonas tumefaciens* Sm. et Town. R. F. Suit et E. A. Eardley, Service de pathologie végétale, Collège Macdonald, P.Q.

La circulation de *Ps. tumefaciens* à travers les vaisseaux du xylem de l'hôte est démontrée. Ce mouvement est influencé par le torrent de la transpiration, le nombre et la dimension des vaisseaux et l'anatomie vasculaire de la tige de l'hôte. Le *Ps. tumefaciens* a existé pendant dix semaines dans le tissu d'internodes apparemment normaux des plants de tomates sans produire de symptômes pathogènes visibles. Les tumeurs secondaires naissant de l'infection vasculaire par *Ps. tumefaciens* sont de trois sortes: (1) tumeurs externes qui naissent des plaies de l'extérieur, et qui rompent les vaisseaux; (2) tumeurs internes aux ruptures dans les vaisseaux du protoxylem; (3) tumeurs faisant saillie qui naissent du xylem sous forme de végétations adventices.

MOULDY CORE IN GRAVENSTEIN APPLES[1]

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Apple growers in the Annapolis Valley are subject to severe losses in some seasons due to the heavy drop of Gravensteins in late August and early September just before the proper date for harvesting this variety. A large proportion of the fallen fruit has an opening in the blossom end leading into the apple and the core region is usually invaded by moulds. These conditions have been known to growers for years and many pick early to avoid losses. The trouble has been brought more forcibly to their attention in recent years as the result of planting and grafting with the red bud sports of this variety, because the apples that tend to drop early develop high colour, and it is from amongst the finest appearing apples on the trees that the loss takes place. The most severe losses recorded were at the average rate of one barrel per tree for a block of 250 trees.

REVIEW OF LITERATURE

It was found in a review of the literature that openings at the calyx end and moulds in the core were well known phenomena. Sorauer (*10*) noted the susceptibility of cores with woolly streaks to attacks by organisms. Longyear (*8*) considered that structural peculiarities rendered certain varieties of apples subject to "blackened seed cavities" and associated a blossom end rot caused by *Alternaria* with calyx peculiarities. A number of workers since have reported the presence of organisms in the cores of apples. O'gara (*9*) in his work on water core of apples mentions *Alternaria* sp. as gaining entrance through cracks. Cook and Martin (*6*) associated "Blossom end and core rot" with *Alternaria* sp. Brooks, Cooley and Fisher (*3*) consider *Alternaria* sp. the most common source of core rots, the entrance of the organism "being favoured by the open calyx that frequently characterizes the apples from irrigated orchards." Heald (*7*) isolated organisms from Stayman Winesap cores and found that *Alternaria* sp. predominated.

Carne, Pittman and Elliott (*4*, *5*), describe four disorders of the variety Cleopatra; namely, woolly streak, hollow core, split core, and mouldy core. They stress the prevalence of split core in years of light crop and consider that these disorders are caused by too rapid and irregular growth. The opinion has been expressed by Brittain and Blair (*1*) and Brittain and Eidt (*2*) that the heavy drop of Gravensteins is connected with open calyx, mouldy core, and low seed content, and that all are correlated with faulty or inadequate pollination. The latter consider that mouldy core is due to the failure of the calyx tube to close and quote Miss Tetley's observations (*11*) that the young apples of the variety Bramley's Seedling have a tube through the style that connects the calyx with the core and that these may or may not become closed during the growth of the apple. They also report Boskoop as affected and point out

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that Gravenstein, Boskoop and Bramley's Seedling are triploids and consider that "This is of interest in view of the observed fact that in triploids, abnormalities are likely to develop late in the life cycle."

Core Characteristics

Woolly Streak.—White woolly streaks extending across the endocarp (hard walls of the core) are very common, particularly in the varieties Gravenstein, Red Delicious, and Ortley (known as Cleopatra in Australia). The endocarp is pulled apart in some cases as much as one-eighth inch and the growth of the cells of the flesh underneath the break causes the white appearance.

Hollow Core.—This is common in a number of varieties. The five carpillary chambers are pulled apart so that a common seed cavity is formed with five more or less shallow lobes. In pomology this has been called abaxile open core.

Split Core.—This is the result of splitting of both endocarp and flesh. Usually such breaks occur at the calyx end, although they may occur at the stem end. Such breaks may reach the surface and result in a passageway into the core. Cases have been found where small breaks became closed, as far as the eye could see, as a result of growth of the surrounding tissue, in the same manner as described in the development of the woolly streaks.

Mouldy Core.—Occasionally an apple is found with a rot developing in the flesh around the core. This is the stage referred to by Carne, Pitman and Elliott (*4*) as "mouldy core"; they consider the presence of organisms not causing a rot as "fungal growth." In the work in Nova Scotia the presence of moulds or discolorations that indicates organisms present has been classed as "mouldy core" by Brittain and Blair (*1*) and Brittain and Eidt (*2*), and this practice is continued because of its acceptance by the growers.

Terms Employed

Some difficulty has been experienced in determining the meaning of certain terms used in describing the calyx end of the apple. The following gives the limits of the terms used in this paper.

1. *Calyx basin.* The depression at the apex of the apple. The sepals divide it from the "calyx tube."

2. *Calyx tube,* sometimes called "Calyx cup." The cavity formed by the union of the bases of the sepals and extending from the sepals to the point of union of pistil and flesh.

3. *Style tube.* A passageway through the centre of the style that connects the core with the calyx tube. This was described by Miss Tetley from the variety Bramley's Seedling.

4. *Open core.* A core with an opening connecting it with the "calyx tube." Includes all breaks in the continuity of the tissue that permit penetration by saprophytic fungi. Replaces "open calyx tube" because it is considered more descriptive and cannot be confused with "open calyx."

5. *Open calyx.* The condition where the sepals do not block the outer entrance to the calyx tube and is contrasted with closed calyx when describing varieties of apples.

Varieties Affected

Table 1 contains a list of varieties that have been personally examined and found affected with mouldy core. There are undoubtedly other varieties which possess this peculiarity but no effort has been made to make a complete survey of the 200 or more varieties available at the Experimental Station, Kentville, N.S.

TABLE 1.—VARIETIES AFFECTED WITH MOULDY CORE IN NOVA SCOTIA

Variety	Chromosome class	Severity of disorder	Comments
Old Gravenstein	Triploid	Severe	Reported by Brittain
Crimson Gravenstein	Triploid	Severe	Reported by Brittain
Banks Gravenstein	Triploid	Severe	Reported by Brittain
Johnson's Gravenstein	Triploid	Severe	Only a few examined
Belle de Boskoop	Triploid	Severe	Reported by Brittain
Bramley's Seedling	Triploid	?	Reported by Brittain
Red Delicious	Diploid	Slight	
Opalescent	Diploid	Slight	Only one large apple observed affected
Stayman Winesap	Diploid	Slight	Reported from U.S.A. by Longyear and Heald
Evangeline	Unknown	Very severe	A local Nova Scotian variety
Ortley (Cleopatra)	Unknown	Severe	Reported from Australia. Probably a diploid.

Object of the Investigation

The main object of the investigation was to find out the cause of mouldy core and although time has not permitted the investigation of all the points raised, a number of interesting facts have been found. The experiments were planned to show the effect on mouldy core of thorough pollination and maximum set, thorough pollination and a light set, thorough pollination and very vigorous growth. Five orchards were studied to secure data under normal conditions. Of these, three were well pollinated and two rather poorly. The suggestion of Brittain and Eidt (*2*) that the triploidy of the varieties affected might have some connection was not supported by finding three known diploid varieties affected. No record of the chromosome number of the variety Ortley was found but the high average number of seeds and high pollen germination strongly indicated that it is diploid. Therefore the fact that Gravenstein is a triploid variety was not considered further.

Experimental Procedure

Experiments in 1933 were carried out in Mr. A. S. Banks' orchard at Waterville, N.S., in a solid block of some 250 trees recently grafted to two red-fruited selections of Gravenstein known as Banks' and Crimson. The variety Crimson Gravenstein was selected for the experiments. One tree was tented and pollinated by using bouquets of Golden Russet bloom and a hive of bees to obtain a maximum set. A second tree had 90% of the blossoms removed and the remaining 500 clusters were hand pollinated using Wagener pollen to obtain a light crop of well pollinated fruit. A third tree whose grafts indicated extreme vigour was hand pollinated to

secure data on the relation of vigour to a full crop. In addition records were obtained from picked, prematurely coloured, and drops from the same orchard to secure data under poor pollinating conditions; from two orchards containing Old Gravensteins at Woodville, one owned by Robert Leslie where the set was moderate and poor pollination might be expected, and one of well-mixed pollinating varieties owned by George McLean, where the set was heavy; also from Old and Crimson Gravensteins on the Experimental Station. The Old Gravensteins were in the Tully orchard which is a block of old trees of many good pollinating varieties and the Crimson Gravensteins were in the main orchard with rows of pollinators adjacent.

Records were kept throughout the season of conditions that might lead to an explanation of cause and factors influencing mouldy core. Apples were tagged on control and experimental trees to obtain the percentage of drop and, with the exception of the grafted tree, the data obtained were in keeping with the results of Brittain and Blair (*1*). The grafted tree was abnormal in its rapid growth, high percentage of abnormal pistils, and very heavy drop in August.

The data obtained in 1933 are not directly comparable in all details with those secured earlier by this department or Brittain and Eidt (*2*). Previously the condition open core meant a visible aperture between calyx tube and core. This year, it was found that an opening might appear closed but brown tissue at the angles of the carpels was an indication that an opening was there through which moulds could enter. For this reason, percentages of open core are higher. Similarly, the percentages of mouldy core are higher because, when cultured, this slightly discoloured tissue, disregarded in 1932, almost invariably gave rise to fungi. The numbers of unfilled seeds were recorded separately but are omitted in this paper because when used it was found that they reduced the correlations between weight and seeds. Brittain and Eidt (*2*) included them.

The Banks orchard had an unusually large number of prematurely coloured fruit, together with a heavy infestation of codling moth and gray-banded leaf roller. The apples attacked by codling moth were rejected because it is a well known fact that such apples colour prematurely and drop early. The impression was obtained that the same might hold true for injuries of the leaf roller type where part of the epidermis is removed. Because of this, insect-injured fruit was rejected, with the exception of two lots. It was necessary to pick 1,599 apples to secure the sample of 1,000 for the control and 767 to secure the sample of 502 for prematurely coloured. The 599 insect injured from the former and the 265 from the latter were examined. The data are presented in Table 2.

TABLE 2.—THE INFLUENCE OF SELECTION ON THE PERCENTAGE MOULDY CORE IN CRIMSON GRAVENSTEINS

Origin	Percentage mouldy core	Average number seeds per apple	
		Healthy	Mouldy
Prematurely coloured	72.7	3.0	2.5
Control	37.4	3.0	2.3
Insect injured from prematurely coloured	60.4	3.1	2.6
Insect injured from control	57.1	3.0	2.5

The percentages of mouldy core in prematurely coloured and insect injured are abnormally high compared with the control and indicate a correlation between them. The reason for the connection between insect injured and mouldy core is not apparent and no attempt is being made to explain it. However, the association complicates the study of the trouble and makes it inadvisable to compare directly orchards with different degrees of pest control.

The Relationship Between Mouldy Core and Pollination

The data from four commercial orchards previously mentioned are presented in Table 3 as indicative of the percentages of mouldy core to be found under ordinary conditions.

TABLE 3.—THE PERCENTAGE MOULDY CORE AND NUMBER OF SEEDS IN PICKED AND PREMATURELY DROPPED APPLES FROM FOUR ORCHARDS

Variety	Orchard	Type of fruit	Percentage of mouldy core	Average number of seeds per apple	
				Healthy	Mouldy
Old Gravenstein	McLean	Picked	10.8	3.3	3.1
		Dropped	47.7	3.7	3.8
Old Gravenstein	Leslie	Picked	23.7	2.5	2.7
		Dropped	64.4	3.4	2.5
Old Gravenstein	Tully	Picked	12.7	3.5	3.7
		Dropped	40.3	3.6	3.8
Crimson Gravenstein	Experimental Station	Picked	11.9	4.2	4.5
		Dropped	26.4	4.5	4.0

The Leslie orchard was the only one that showed signs of inadequate pollination, and the data resemble in many respects those secured from the Banks' orchard (see Table 4). The high percentage of mouldy core and low seed content is quite in keeping with the theory advanced by Brittain and Eidt (*2*). The owner, however, has never considered the premature drop as being of economic importance. The McLean orchard had a very small drop and it was not possible to find the one thousand prematurely dropped apples desired for the records. The Tully orchard had a moderate drop and the Station orchard had a heavy one, although judging from the seed content these orchards were equally as well pollinated as the McLean orchard.

Table 4 presents the data for the different pollination methods.

In Table 4, the figures for "Control" were obtained by taking 100 apples from each of ten trees. Five of these were grafts and five, normal trees. The mouldy core averaged 38.6% on the grafts, and ranged from 32 to 48; on the normal trees 36.2%, and ranged from 22 to 53. The averages obtained from the drops from the experimental trees were obtained from 44 apples for the grafts, 50 for the deflorated and 111 for the tented. The definite association established by Brittain and co-workers between methods of pollination and seed content is evident. It cannot be concluded, however, that adequate pollination controls mouldy core. The

TABLE 4.—THE INFLUENCE OF DIFFERENT METHODS OF POLLINATION ON THE AMOUNT OF MOULDY CORE AND THE NUMBER OF SEED IN PICKED AND DROPPED APPLES. ORCHARD OF A. S. BANKS

Variety	Tree treatment	Method of pollination	Type of fruit	Percentage of mouldy core	Average number of seeds per apple	
					Healthy	Mouldy
Crimson Gravenstein	Tented	Bees and bouquets	Picked	22.2	4.2	4.4
Crimson Gravenstein	Tented	Bees and bouquets	Dropped	53.2	4.2	4.6
Crimson Gravenstein	Deflorated	Hand	Picked	23.3	6.0	6.0
Crimson Gravenstein	Deflorated	Hand	Dropped	34.0	4.2	6.0
Crimson Gravenstein	Grafts	Hand	Picked	38.4	4.7	4.1
Crimson Gravenstein	Grafts	Hand	Dropped	29.3	4.8	5.1
Crimson Gravenstein	Control	Open	Picked	37.4	3.0	2.3
Crimson Gravenstein	Control	Open	Dropped	68.8	2.6	2.9

percentage from the tented tree is within the extremes of mouldy core from the controls. It should be stressed that mouldy core was very slight in most of the apples from the tented tree and undoubtedly many would have been overlooked in 1932.

Most of the data from Tables 3 and 4 have been arranged in Table 5 to show the percentage of mouldy core in different seed content classes. It was felt that such a treatment should show any relationship that existed between seed content and mouldy core.

TABLE 5.—THE RELATION BETWEEN SEED CONTENT AND MOULDY CORE IN APPLES

Variety	Origin	Type of fruit	Per cent of mouldy core in different seed content classes			
			0–2	3–5	6–8	9–12
			%	%	%	%
Crimson Gravenstein—A. S. Banks	Tented	Picked	19.0	21.2	25.4	44.4
	Deflorated	Picked	26.6	23.6	23.2	20.5
	Grafts	Picked	33.0	36.4	41.0	—
	Orchard run	Picked	45.0	32.4	13.5	—
	Orchard run	Dropped	70.9	67.7	58.0	—
	Premature	Picked	75.3	71.0	69.4	—
Old Gravenstein	Orchard run (Leslie)	Picked	22.2	17.2	50.0	—
	Orchard run (Leslie)	Dropped	65.4	63.8	58.1	—
	Orchard run (McLean)	Picked	10.5	6.4	8.7	—
	Orchard run (McLean)	Dropped	44.7	44.1	49.6	—
	Orchard run (Tully)	Picked	10.4	13.3	15.2	17.3
	Orchard run (Tully)	Dropped	34.1	42.4	41.3	—
Mean of seed content classes			42.2	33.2	32.1	25.3

It is at once evident that there is no constant trend of the percentages of mouldy core from the various orchards and treatments. It is apparent from a study of the experiment treatment that factors other than pollination play a part, for the trends are reversed in defloration and grafts. However, the means of the total apples for each class support the theory of a connection between low seed content and mouldy core. It must be concluded, therefore, that in certain orchards there is a definite association between low seed content and mouldy core that does not appear in others and that good pollination tends to reverse this relationship.

The Relationship Between Weight and Mouldy Core

While taking the records of seeds and mouldy core it was noted that the larger apples appeared to have a higher percentage of mouldy core and as a result the rest of the fruit was weighed to the nearest gram. It was found that mouldy core apples averaged 12.2 grams heavier than healthy with a range of from 3 to 17 grams per tree.

The data for weight and mouldy core, arranged in the same manner as for seeds and mouldy core, are presented in Table 6.

Table 6.—The relation between weight and mouldy core in apples

Variety	Origin	Type of fruit	Per cent of mouldy core in different apple weight classes, grams			
			0–70	71–100	101–130	131 and over
			%	%	%	%
Crimson Gravenstein	Tented	Picked	12.8	20.0	30.5	37.7
A. S. Banks	Tented	Dropped	21.9	59.0	88.8	—
	Deflorated	Picked	—	0.0	15.8	26.9
	Grafts	Picked	—	20.0	35.0	41.8
	Orchard run	Picked	0.0	18.7	18.7	32.2
Crimson Gravenstein	Orchard run	Picked	—	2.8	14.0	17.4
Exp. Station	Orchard run	Dropped	—	10.0	20.5	50.0
Old Gravenstein	Orchard run	Picked	1.3	6.2	13.7	23.3
Exp. Station	Orchard run	Dropped	28.6	32.1	45.2	64.3
Mean of apple weight classes			9.7	16.0	20.2	29.6

The classes with the exception of the first and last roughly corresponded to quarter-inch differences in diameter of the apples. It is concluded from Table 6 that mouldy core is associated with size of the fruit.

To find out whether there was any connection between seeds and weight the correlation coefficients have been calculated from the data available from the larger population. It is regretted that weights are not available for apples from all the orchards. Those available are presented in Table 7.

There is a low positive correlation between filled seeds and weight that is significant. This correlation was greatly reduced or insignificant when weight and all seeds were correlated and it was because of this that filled

TABLE 7.—CORRELATION COEFFICIENTS FOR WEIGHT OF APPLE AND NUMBER OF FILLED SEED IN HEALTHY AND MOULDY-CORE APPLES FROM VARIOUS SOURCES

Variety	Origin	Type of apple	Condition of apples	Number of apples	Correlation coefficient
Crimson Gravenstein	Tented	Picked	Healthy	749	0.247 ± 0.023
A. S. Banks	Tented	Picked	Mouldy	214	0.201 ± 0.044
	Tented	Picked	Healthy*	749	0.108 ± 0.024
	Tented	Picked	Mouldy*	214	0.162 ± 0.045
	Hand pollinated	Picked	Healthy	311	0.337 ± 0.034
	Hand pollinated	Picked	Mouldy	123	0.147 ± 0.060
	Control	Picked	Healthy	153	0.294 ± 0.050
Old Gravenstein	Tully orchard	Picked	Healthy	2751	0.363 ± 0.011
		Picked	Mouldy	400	0.368 ± 0.029
		Drops	Healthy	371	0.355 ± 0.031
		Drops	Mouldy	250	0.365 ± 0.037

*Analysis based on all seeds.

seeds were used throughout. The correlations are similar for healthy and mouldy, as shown in the analysis of the data from the Tully orchard. The low correlation for hand pollinated to mouldy is due to the average weight of the grafted tree being 141, and of the deflorated being 153 grams. The healthy fruit was almost identical.

Organisms Present

The results from isolations made in 1933 were similar to those previously obtained and are briefly summarized as follows. The genus *Alternaria* predominated. The cultures differed so much in growth characteristics that eight types could be distinguished. However, the spore sizes were uniform for all and fell within the limits of *Alternaria tenuis* Nees, a common saprophyte. The genus *Fusarium* was also well represented by cultures of ten distinct types. Other genera were represented by a few cultures of *Cladosporium*, *Penicillium*, and unknown sterile forms. Yeasts and bacteria were isolated quite commonly with the various moulds, as would be expected with a visible opening present. The results of the two years' isolations indicate that the organisms associated are chance invaders which thrive according to their adaptability to this particular environment.

Observations of the Calyx End of the Apple

An attempt has been made to ascertain the manner in which an open core originates. Several papers have dealt with the vascular system of the apple, but none has described the development of the calyx region with sufficient detail to serve as a basis for an explanation of the problem. Tetley (*11*) describes the style tube as becoming closed by interlocking of the cells, and this is the view accepted by Brittain and Eidt (*2*) in respect to Gravensteins. During the present studies a style tube has been found in Bramley's Seedling but not in Gravenstein. During the examination of the variety Red Delicious, a few open cores were found that might have originated in the same manner as in Bramley's. These observations

are based on the similarity of the condition in mature apples, and a study of the seasonal development may show otherwise.

A limited study was made of young developing fruit on the only material available which, unfortunately, was on the unsusceptible variety Wagener. Samples of this variety, preserved for another purpose, had been collected at weekly intervals starting with the pink bud stage and ending when the apples were one and a half centimetres in diameter. Freehand sections were made and it has been impossible to demonstrate any opening into the carpels in this material either through the style or where the style unites with the tissue of the young apple. The tissues of the flesh and pistil are continuous in the youngest stages. In apples one and a half centimetres in diameter openings in the tissue are visible near the carpels. Their origin is not known. In mature Wageners, openings at irregular points in the interior of what was originally the style were found. In the variety Stark several apples were sectioned and such openings were not found. A study of this region in varieties affected with open core shows a number of interesting conditions. In Red Delicious and Ortley the growth of the apple frequently causes a pulling apart of the strands of the old style. This also happens in many varieties not affected with mouldy core, such as McIntosh. The difference is that the separation of the strands of the style causes a rupture that reaches the calyx tube in Delicious and Ortley and does not in other varieties.

There is great variation between varieties in the development of the flesh of the apple around the calyx end. In Gravenstein the growth gives rise to a wide basin and a large tube that varies in depth from direct contact with the carpels to being one centimetre away. In Stayman Winesap the apex of the apple is more pointed and the calyx tube is close to or in contact with the carpels. In certain apples of these two varieties there has been little or no development of the tissue that was in direct contact with the style at blossom time and the calyx tube has not been separated from the carpels by the growth of this tissue. In the variety Stark, for example, not affected with open core, the same region has increased until there is often over one centimetre of flesh.

Split core was commonly found in three varieties. In Gravenstein it varied from tiny breaks at the apex of the carpels to cracks that almost divided the apple in half, longitudinally. In Ortley the splits were longitudinal or transverse. In Stayman Winesap they were transverse and occurred most frequently at the base of the carpels. Breaks in the flesh occur in other varieties not affected with open core, such as McIntosh which is subject to hollow core and the pulling apart of the strands of the style.

Excluding Bramley's Seedling and its style tube, open core is considered as arising as a result of rapid or irregular growth during the summer which sets up stresses that cause ruptures in the different tissues. These are in order of decreasing frequency: woolly streak, hollow core, split core, and open core. The type of disorder is dependent on the peculiarity of the variety. The amount of open core may be modified by any of the factors influencing rate of growth and size of fruit, such as set of fruit, vigour of the tree, water supply, fertilizer, etc.

DISCUSSION

Although not presented, the data obtained in 1933 show conclusively the close relationship between mouldy core and open core. A few healthy open core, and a few closed mouldy core fruits were found in McLean's orchard. The other orchards and experiments had very few or no apples in these categories. The study of the organisms present indicates that they are casual invaders by way of the opening and that *Alternaria* and *Fusarium* species thrive because of their adaptability to the peculiar environment of the core of the apple.

The relationship of premature colouring of the fruit to open and mouldy core is not clear because 25 to 40% of the prematurely coloured are free from the trouble. Therefore it seems better to think of the factors that cause premature colouring of the fruit as predisposing it at the same time to open core and mouldy core. Brittain and Blair's work (*1*) proves a very close relationship between pollination as modified by weather and orchard conditions and average seeds per apple. Studying the average seed content of the different classes it is to be noted that no consistent differences are to be found between mouldy core and healthy, between premature coloured and healthy, or between premature drops and picked.

Healthy picked and healthy drops in the Tully orchard had lower average seed contents than the mouldy apples in the same classes, and yet significant differences are reversed in Banks' orchard where the healthy picked had an average of 3 filled seeds and the mouldy, 2.3. It is evident that the average seed content cannot be taken as evidence to support a pollination theory of the origin of mouldy core and premature colouring of the fruit. The data more consistently supports the theory held by Carne, Pittman and Elliott (*5*) that "split core" which may become open core is the result of rapid and irregular growth.

Mouldy core (which follows open core) apples always averaged heavier (larger) than healthy. The largest amounts of mouldy core were found in orchards with a light to medium crop. Even this is not completely supported by the records from individual trees, for the tented tree had 22% mouldy core, which was the same as for one open-pollinated tree in the same orchard. Individual trees vary greatly and it is felt that the high percentage found in the tented tree is the upper limit of mouldy core in a heavy crop. It is evident that the variety Gravenstein is predisposed to open and mouldy core from its growth habits and the constitutional weakness of the calyx end.

When considering the evidence in favour of effective pollination preventing the premature drop associated with mouldy core, we have the tented tree, deflorated tree, McLean's orchard and the Tully orchard with few drops, in support. On the negative we have the recently grafted tree, hand pollinated tree and the Crimson Gravensteins in the mixed orchard of the Experimental Station with many drops where conditions for effective pollination existed. If we use the average number of filled seeds per apple to indicate degree of pollination we have conflicting evidence The group in support of the pollination theory all have a high average seed content (3.7 to 6.0 per cent), but on the negative we have the Experimental Station Crimson Gravenstein with an average of over 4 filled seeds

per apple, which is identical with that of the tented tree, and yet with a drop comparable to Banks' open pollinated with an average close to 3 filled seeds. The average filled seed this year is very high, in many cases as high or higher than total seeds as reported by Brittain and Eidt (*2*). Weather conditions were favourable for pollination but in A. S. Banks' orchard bees were almost totally absent up to and during full bloom of the Gravenstein. Whether this obscures results or not cannot be stated. The general crop and drop bear the same relationship to each other as in 1932.

SUMMARY

1. The percentage of mouldy core in Gravensteins is directly dependent on the percentage open core. The various fungi present are considered chance inhabitants that rarely parasitize their host.

2. Mouldy core apples average heavier than healthy and on any one tree the percentage of mouldy core is higher in the heavier apples.

3. Open core is considered the result of rapid growth at some period during the life history of the apple that causes a break in the tissues of the core that leads to an opening, usually into the calyx tube.

4. The early maturity (premature colouring) of the fruit is closely related to mouldy core and open calyx, but 25 to 40% cannot be explained, as they are free from the trouble.

5. It has not been found possible to correlate average seed content with mouldy core, or premature drop under 1933 conditions.

6. It is shown that faulty pollination cannot be considered the fundamental cause of mouldy core. It is an important factor as it influences the number of apples that set, and the set influences the size to which the individual apples grow.

7. Premature drop is considered as partially correlated with premature colour which indicate early maturity. Early matured apples are 60 to 75% open calyx mouldy core, due, it is considered, to too favourable chances for growth.

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Résumé

Moisissure du cœur dans les pommes Gravenstein. K. A. Harrison, Laboratoire fédéral de pathologie végétale, Kentville, N.-E.

Le pourcentage de cœur moisi dans les Gravensteins dépend directement du pourcentage de calices ouverts. On considère que les différents champignons présents sont des habitants de hasard, qui attaquent rarement leur hôte. Les pommes à cœur moisi sont en moyenne plus lourdes que les pommes saines et sur tous les arbres le pourcentage de cœur moisi est plus considérable dans les pommes lourdes que dans les autres. On considère que le cœur ouvert est le résultat de la végétation rapide à une certaine période pendant le cycle évolutif de la pomme qui cause une rupture dans les tissus du cœur, conduisant à une ouverture, généralement dans le tube du calice. La maturité précoce (coloration prématurée) des fruits se rattache intimement au cœur moisi ainsi qu'au calice ouvert, mais de 25 à 40 pour cent ne peuvent s'expliquer, car ils n'ont pas cette infection. On n'a constaté aucune relation entre la proportion moyenne de pépins avec le cœur moisi, ou la chute prématurée des fruits dans les conditions de 1933. Il a été démontré que la pollinisation défectueuse ne peut être considérée comme la cause fondamentale du cœur moisi. C'est un facteur important car il influence le nombre de pommes qui nouent et la nouaison influence la grosseur que les différentes pommes peuvent atteindre. On considère que la chute prématurée a certains rapports avec la couleur prématurée qui indique une maturité hâtive. De 60 à 75 pour cent des pommes mûries prématurément ont un cœur moisi et un calice ouvert dont la cause est due, croit-on, à des chances de végétation trop favorables.

COLLETOTRICHUM GRAMINICOLUM (CES.) WILS. AS A PARASITE OF THE STEM AND ROOT TISSUES OF *AVENA SATIVA*[1]

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During recent years, oats *(Avena sativa)*, in the Edmonton district, have been observed to suffer from a rather serious type of root rot, the effects of which are most evident just prior to elaboration of the adventitious root system. This report will provide evidence, secured since the preliminary note (*4*), that *Colletotrichum graminicolum* (Ces.) Wils. may cause the symptoms referred to.

Symptoms

Gross symptoms of the disease in the field appear about six to eight weeks after seeding, before the adventitious root system can provide food. In severe cases, rather well defined areas occur, where the plants are distinctly stunted (Figure 1A). Such areas, however, are not necessarily those in which stunting, caused by malnutrition of the plants, are found, although indications are that both disease and malnutrition may appear together, the effects of one apparently intensifying those of the other.

The first and second leaves of affected young plants are more or less brown, becoming reddish brown at first and finally dying from the tip toward the base. Chlorotic areas and disease lesions are normally absent. In Figure 1B are shown affected oat plants which are typically thin in root, stem and general growth habit. These were grown in the greenhouse in natural field soil from an infested area. In severe cases the emergence of the panicle is retarded and definitely reduced in size, and the numbers of tillers is seldom more than one. Although infection at the base of the panicle has been observed in the field (Figure 2, A and B), and also produced experimentally, it is uncommon. However, infection of any part of the stem may, under moist conditions, become heavy late in the season. Usually this is greater below the nodes than above them, apparently because these areas are exposed. The seminal roots and the first internode of affected plants early become thin, poorly developed, and straw coloured. In general, the affected parts do not become dark as when roots of cereals are infected by *Ophiobolus graminis* or *Helminthosporium* sp. and other common root-rotting organisms. The shrinkage of the seedling axis from the scutellar node to the coleoptilar or second node, is distinct and typical. Although adventitious roots may be attacked as they emerge, there is a marked tendency for these to escape serious injury. When these roots are established the plant begins to recover.

Soon after the tendency toward recovery is evident, black, stromatic bodies, in the beginning loose and very small, later becoming more solid, and in shape varying from round to long oval, appear on the rhizome (Figure 2C), and also on the culm at ground level. These bodies persist and are viable at least until May the following spring. Careful, although

[1] Contribution No. 420 from the Division of Botany, Fxperimental Farms Branch, Department of Agriculture, Ottawa, Canada, co-operating with the University of Alberta. Presented at the fourth annual meeting of the Canadian Phytopathological Society held at Macdonald College, P.Q., June 26 and 27, 1934.

[2] Plant Pathologist in Charge.

not exhaustive, search has so far failed to reveal spores produced on these bodies borne below ground level.

As far as could be determined, the grains from plants, the culms of which bore rather heavy infestation on the higher nodes and stems, were not affected, although it would seem that such attack, if early and severe, might lead to some shrinkage in yield. However, indications were that severe infection of the crown of oats at soil level hastens maturity of both plant and grain. The main damage seems to arise from injury to the seminal roots, and to the seedling axis below the coronal node during the seedling stage, which takes form in reduced size of panicle, number of tillers, and stunting mentioned earlier.

Prevalence

This disease now appears to be prevalent in the black soil area immediately north and east of Edmonton. It has also been observed in the Peace River district, and at other points in the black soil area tributary to Edmonton, and southward for nearly 200 miles. Bodies, typical of *C. graminicolum*, on the base of mature wheat plants collected by Dr. P. M. Simmonds at Indian Head, Saskatchewan, were identified. With regard to the differential effect of previous crops on the disease, no definite evidence is available, but it is interesting to note that several of the most severe cases have followed two or more crops of oats.

Historical

In 1909 Selby and Manns (*6*) of Ohio published a study on a new anthracnose disease of cereals and grasses, and described the causal fungus as *Colletotrichum cereale* Manns, parasitic on the roots, culms, blades and spikes of certain cereals, including *Triticum vulgare*, *Avena sativa*, *Hordeum vulgare*, *Triticum spelta*, *Dactylus glomerata*, *Phleum pratense*, *Poa pratensis*, and *Bromus secalinus*. Apparently the infection of the root and basal portion of the hosts mentioned was not determined by experiment, but based on field observations. In view of the possible effect of other pathogenic soil-inhabiting fungi, such as *Fusarium* spp. and *Helminthosporium* spp., it would seem unsafe to conclude from the mere presence of acervuli that the root rot observed was caused by *C. graminicolum*. However, their experimental results leave no doubt that this fungus is pathogenic to the above-ground parts of a number of the Gramineae.

In Canada, this species has been reported (*1*) on certain hosts, including Poa, Beckmania, wheat and oats, but apparently it has not been considered an important root-rotting organism.

The Pathogen

A number of isolations were made from the young seminal roots, the seedling axis below the crown, the crown, and also from acervuli, or sclerotoid bodies, produced on parts in the field. A *Colletotrichum* sp. was a frequent isolate. Two of these isolates from the first internode were kindly identified as *Colletotrichum graminicolum* (Ces.) Wils. (*C. cereale* Manns) by Mr. S. F. Ashby, Mycologist, Imperial Mycological Institute, Kew, England.

EXPERIMENTAL RESULTS

Pathogenicity

Tests were made in open pot culture, using one of the identified isolates, increased on a sterilized mixture of soil and oat hulls. This was mixed with sterilized soil, and grains of oats, of both Victory and Banner varieties, surface disinfected in mercuric chloride, were planted. The plants from certain pots were taken up at intervals of 10 days, examined for disease, and tissue reserved for microscopic examination.

There was no doubt that the culture used had severely disabled the seminal roots and injured the first internode of the young oat plants in a way corresponding to the gross symptoms observed in the field. That is to say, these seminal roots were more or less dead and stunted, and usually straw-coloured, while the rhizome was definitely shrunken from the scutellar node to the coronal node. As soon as the adventitious roots were established, the plants tended to revive, and many became normal under the conditions of the test (soil temperature 19°–21° C.). In pot culture, faint beginnings of acervuli appeared about 40 days after seeding, and in 60 days on the internodes and the crown at or below ground level. Indications were that the seminal roots are particularly susceptible, as is also the first internode. As a rule, the adventitious roots were not severely injured.

Pathological Histology

Portions of the seminal and adventitious roots of the first and second internode and of the crown area were fixed for 24 hours in Bouin's fluid, to which one gram of urea was added for each 100 cc., washed in four changes of 30% alcohol, and dehydrated by the N-butyl alcohol method, then embedded in paraffin containing 0.001% crude rubber. The sections, cut 15 μ in thickness, were stained in 1% aqueous safranin for one minute, rinsed in tap water, then placed in picro-aniline blue, as prepared by Cartwright (*2*) for 20 minutes, rinsed, and dehydrated in 95% and absolute alcohol, followed by clove oil, then mounted in balsam.

Seminal roots

The seminal roots of affected plants were extensively invaded soon after they were produced. The cells of the cortex and endodermis were collapsed and the invading hyphae more or less completely disintegrated. The cells of the pericycle and central stele, although ramified, retained their form, and the hyphae appeared to persist. In fact a clear cut case of disintegration of hyphae was not observed in either the pericycle or the stele. The cells of the pericycle were obviously and characteristically plugged by hyphae arranged parallel to the main axis of the root. The stele, although penetrated much more slowly than the cortex is, may be extensively ramified and more or less plugged. The invasion of a seminal root 10 days old by *C. graminicolum* is illustrated in Figure 3A. With young seminal roots so invaded and obviously incapacitated in so far as their absorbing area is concerned, and with the vessels only partly functional, the observed injury reflected by such young seedling plants is not surprising.

FIGURE 1—*Avena sativa* attacked by *Colletotrichum graminicolum.* A. Typical effect of injury to root and rhizome in field. Left, seedling; right, post seedling stage. B. Left, thin appearance of plants reproduced in greenhouse in infested soil from field; and right, normal check plants. C. Typical sclerotoid bodies on plants 60 days old, grown in sterilized soil artificially infested.

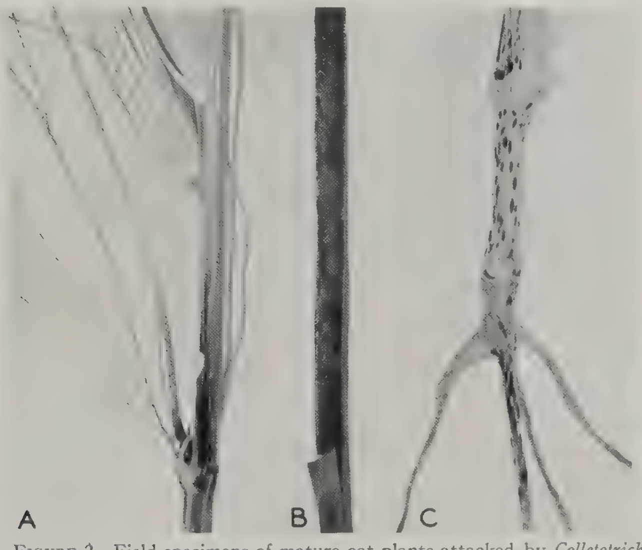

FIGURE 2—Field specimens of mature oat plants attacked by *Colletotrichum graminicolum.* A. Infection of panicle and peduncle, exterior view. B. Sclerotoid bodies within cavity of stem. C. Sclerotoid bodies on rhizome and base of culm.

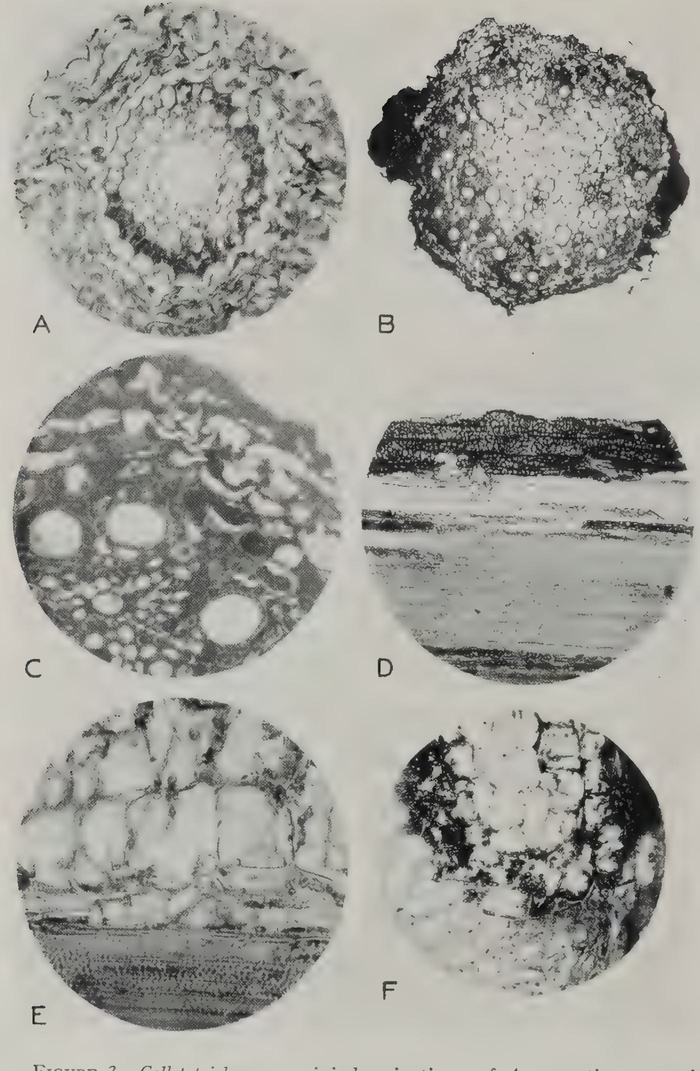

FIGURE 3—*Colletotrichum graminicolum* in tissue of *Avena sativa* grown in sterilized soil artificially infested. All tissue, excepting that of A, is 60 days old. A. Seminal root of seedling plant 9 days old. Note typical collapse of cortex and endodermis, with attendant disintegration of hyphae, also the extensive development of large persisting hyphae in the pericycle, and the beginning of invasion of the central stele. B. A cross section of first internode extensively ramified by hyphae and typical sclerotoid bodies on periphery. C. High magnification of a part of vascular bundle in B. showing collapsed cortex and extensive penetration and plugging of all cells of bundle, excepting the large vessels. D. A tangential long section of rhizome, with sclerotoid bodies on periphery, and sclerotoid and non-sclerotoid mycelium lengthwise of cells. E. Pith tissue of rhizome extensively penetrated. F. A long section through the hollow central cavity of stem immediately above crown node. Note large thick walled hyphae above and extensive penetration of tissue below.

The rhizome

The first internode of the rhizome was, as a rule, easily and extensively invaded (Figure 3, B, C and D), but probably not as quickly or as completely as the seminal roots were. In severe and early cases there was complete collapse of the cortex, accompanied by disintegration of the hyphae. However, penetration of the cortex and epidermal cells of the older tissue is slower than it is in young tissue, and both cells and hyphae tend to remain intact longer. The vascular bundles were often extensively penetrated, but, as a rule, this tissue offered considerable resistance. In the pith cells (Figure 3E) the hyphae developed profusely, and did not disintegrate readily.

Coronal node

From certain rows of infected cells located just below the cortex of the rhizome, the hyphae passed the coronal node, and thence up the culm, and also into the central cavity of the stem immediately above the coronal node, where the mycelium developed vigorously (Figure 3F), and frequently extended rather deeply into the adjacent functional tissue. Infection of the latter cavity was sometimes effected directly through the coronal node, the hyphae passing by way of the softer tissue. However, due to the resistance offered by the vascular strands of this area, progress was slow.

Adventitious roots

In general, the adventitious roots below ground level escaped serious injury, although there were individual cases in both greenhouse and field material where *C. graminicolum* entered deeply with effect. Penetration was usually deeper in the angle made by the root with the crown on the superior side than it was below. Root and also shoot primordia emerging from invaded areas of the crown were frequently damaged. In these roots, as in the seminal roots and rhizome, the hyphae tend to mass in certain continuous rows of cells just below the cortex. Acervuli seldom formed on the surface of the secondary roots below ground, but commonly did so when they were exposed.

Stem

Acervuli devlop most conspicuously on the lower part of the culm, especially on the exposed area just below the node, which is unprotected by the leaf sheath. These bodies break through in the depressions bearing the stomata. In certain cells just below the hypoderm the fungus may pass some distance up the culm, usually plugging them or forming extensive sclerotoid masses near the surface. The conducting tissue of a stem approaching maturity, although often penetrated extensively, offers considerable resistance to radial penetration. However, the hyphae soon reach the central cavity of the stem. This is usually between the large vascular bundles, where the hypoderm band is thinnest and the ground tissue widest. Bodies, which are apparently identical to those which occur on the exterior surface, soon form. Apparently these bodies within the stem cavity bear spores very rarely, if at all, since none were observed in the many specimens examined.

Penetration phenomena

The hyphae of *C. graminicolum* vary greatly in width, and in thickness of the cell wall, as do the hyphae of several well known plant pathogens when in the tissue. However, its general appearance and behaviour differ

sufficiently from that of *O. graminis*, *H. sativum* and *Fusarium* sp. for its identification. One outstanding feature is the extensive packing of the cells in certain areas with more or less sclerotoid mycelium (Figure 3D).

The invading hyphae may be very small threads, or very large and swollen with protoplasm. On penetrating a cell of the epidermis or cortex, a hypha commonly swells to fill a large part of the cell, and extends close to the wall, pushing the contents aside, without causing apparent injury. This behaviour was quite marked in the long epidermal cells. On reaching the end wall of the cell the hypha may pass through it, or curve back to the opposite end, where it again turns, and finally fills the cell with two or more strands. Later, before the cell is killed, these strands may or may not become closely septate, with thick walls, and persist. Again, large hyphae were commonly observed to pass without apparent difficulty through the end walls of several long cells in a row. The thin cells of the cortex of seminal roots, and those of the first and second internode of the rhizome, are penetrated rather easily and freely. Progress of the hyphae in a radial direction is definitely hindered by the sterome and vascular elements, and particularly by the large vessels. However, even these large vessels are entered, but apparently not plugged. There is a distinct tendency for the hyphae to plug sterome cells.

Leach (*3*), studying the behaviour of *C. lindemuthianum* in the tissue of *Phaseolus vulgaris*, observed two kinds of hyphae, namely large primary, which were confined to relatively few cells, and which were compatible with the cytoplasm and nucleus for some time; and secondary or smaller hyphae, which were derived from the primary form and which passed through the tissue freely and rapidly in all directions. He states: "It is quite evident that the difference in character of the primary and secondary mycelium are due to the differences in resistance offered by the cell walls.", which, he adds "depends largely on the age of the tissue." In the present study both large and small hyphae were observed, but whether this difference was due to age of tissue or to the kind and amount of food, or to the softening of the tissue by enzymes elaborated by the primary mycelium was not determined.

Habitat

Experimental results leave no doubt that *C. graminicolum* is a typical soil-inhabiting fungus. Sclerotoid bodies of *C. graminicolum* were observed on the stubble of wheat and of oats plowed under one and two seasons previously, and the fungus isolated therefrom. The possible relation of this as a source of infection to subsequent crops was not determined. However, tests made in open pot culture, and also pure culture, indicated that the isolate of *C. graminicolum*, used in these experiments, does not attack wheat, barley or flax. This suggests the possibility of races of *C. graminicolum* specific to oats, as well as to wheat. Varietal differences in resistance and the host range were not studied.

DISCUSSION

Since the results of this study are based on the performance of a single isolate of *C. graminicolum* in open pot culture, and, therefore, no account was taken of possible physiologic forms, too definite conclusions cannot be

drawn regarding the pathogenicity of this fungus to oat plants under field conditions. Moreover, in either field or artificial pot culture the association effects (*5*) of other soil micro-organisms, undoubtedly play an important role in the development of the disease. However, under the conditions of the test, fairly typical symptoms of the disease, as it appears in the field, were reproduced in the greenhouse. Also, microscopic studies on field and greenhouse material gave unmistakable evidence that *C. graminicolum* could cause definite injury to seedling plants, and to some extent injure more mature ones.

Apparently the cortex of the young seminal roots, and that of the stem between the scutellar and crown nodes, is, of all tissue, most easily and severely affected. Indeed, with the exception of a transient compatibility of host and pathogen, there is complete and early collapse of the cells of the cortex and endodermis with attendant disintegration of the invading hyphae. In general, penetration of all parenchymatous cells is free and relatively easy, especially those with thinner cells, which includes the pith. While some resistance is offered by the sterome and the elements of the vascular bundles, all are penetrated, and, with the exception of the large vessels, are, as a rule, plugged. In both cases the cells remain apparently intact, and the hyphae do not disintegrate, which interesting behaviour is in striking contrast to that which occurs in the cortex, and for which no satisfactory explanation seems available. Thus, on one hand there is the definite, although transient, compatibility between hyphae and host cells when the cortex is first penetrated, followed by collapse of the cells and disappearance of the hyphae, and, on the other hand, the case where the cells apparently are not injured and do not collapse or the hyphae disintegrate. Leach (*3*), in a study of the parasitism of *C. lindemuthianum* on *Phaseolus vulgaris*, states: "In the early stages of normal infection of a susceptible host, *C. lindemuthianum* obtains its nourishment from the living host protoplast.", and observes that in the more resistant tissue the mycelium disintegrates, killing the host protoplast, and staining the cell wall and its contents. He interprets the phenomenon on a nutritional basis, the hyphae being destroyed by autolysis, induced by starvation. If the foregoing theory also applies to the behaviour of *C. graminicolum* in the cortex of *A. sativa*, may one conclude that the vascular and mechanical elements, which are more resistant to penetration, were able to supply sufficient food to prevent autolysis? Hence, in this case it would be conceivable that the antecedents for hyphal lysis occur at least more readily in tissue, the cells of which have functional nuclei and cytoplasm, than in those without.

With regard to the disease as it appears on seedling plants under field conditions, the evidence secured by this study indicated that the early browning of the roots, and stunting, mentioned, is caused mainly, if not wholly, by the practical destruction of the seminal roots, and severe injury of that part of the stem below ground level. On the other hand, the tendency toward subsequent recovery of such plants undoubtedly is explained by the fact that it is coincident with the development of effective adventitious roots, which, as a rule, are not severely penetrated.

ACKNOWLEDGMENT

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Résumé

***Colletotrichum graminicolum* (Ces.) Wils. Parasite des tissus de la tige et de la racine de *Avena sativa*. G. B. Sanford, Laboratoire fédéral de pathologie végétale, Edmonton, Alberta.**

Les observations qui ont été faites en plein air, de même que les études conduites en serre et au laboratoire, ont démontré que *Colletotrichum graminicolum* peut créer une grave infection parasitaire des racines séminales et des internodes de la tige au-dessous du collet de plants de l'avoine ordinaire, et causer ainsi différents degrés de rabougrissement et de détérioration, et réduire le nombre des talles et la dimension de la panicule. En général, les plants attaqués tendent à se remettre après que les racines adventices sont établies. L'historique pathologique des racines séminales, du rhizome, du node coronal, des racines adventices et de la tige est décrit ainsi que les phénomènes de pénétration. La culture employée n'a pas attaqué la base ni les racines du blé, de l'orge ou du lin, quoique la présence de cette espèce ait été constatée sur le blé.

STUDIES ON THE CONTROL OF ROOT-ROT DISEASES OF CEREALS CAUSED BY *FUSARIUM CULMORUM* (W.G. SM.) SACC. AND *HELMINTHOSPORIUM SATIVUM* P., K., AND B.

II. PATHOGENICITY OF *HELMINTHOSPORIUM SATIVUM* AS INFLUENCED BY *CEPHALOTHECIUM ROSEUM* CORDA IN GREENHOUSE POT TESTS[1]

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INTRODUCTION

Numerous studies have been made on the association of micro-organisms, not only culture but also in the soil. Experiments to determine the effects of certain soil-inhabiting micro-organisms on the development of diseases caused by fungal pathogens are reported by Johnson (*12*), Endô (*4*), Bamberg (*1*), Weindling (*21*), and others. The results have indicated that many plant diseases caused by soil-borne fungi are influenced by associated organisms to a significant degree, not only as to retardation, but also as to acceleration, of disease processes. The importance of investigations on the effects of known mixtures in comparison with the effect of individual organisms alone in their relation to plant disease has been adequately discussed by Fawcett (*5*), who gives a complete review of the literature upon the subject.

During the past few years considerable attention has been given to the relation of biological antagonism to infection of cereals by root-rot fungi. Henry (*11*) and Sanford and Broadfoot (*19*) found that the microflora of the soil had a marked inhibitive action on the development of root-rot diseases of wheat caused by *Ophiobolus graminis* Sacc. and *Helminthosporium sativum* P., K., and B. In a study of fungi isolated from Manitoba soil Bisby, James and Timonin (*2*) found that *Trichoderma lignorum* (Tode) Harz plays an important part in suppressing the virulence of *Fusarium culmorum* (W.G. Sm.) Sacc. and *Helminthosporium sativum* on wheat. Factors affecting the pathogenicity of cereal root-rot fungi, and the importance of the factor of biological antagonism in the cereal root-rot problem have been discussed by Garrett (*8*). The present paper gives the results of experiments designed to determine the effect of *Cephalothecium roseum* Corda on the pathogenicity of *Helminthosporium sativum*, and gives a detailed account of the methods employed in pot-culture studies with cereal root-rot fungi under greenhouse conditions.

EXPERIMENTAL METHODS

The difficulty of controlling the soil environment in greenhouse pot-culture tests with cereal root-rot fungi is generally recognized. Garrett (*8*) examined the available published work on the effect of soil temperature and moisture upon infection by cereal root-rot fungi and found that in many cases the influence of these factors upon infection varied in an unaccountable manner. The absence of uniformity in the results was

[1] Contribution No. 412 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Ontario, Canada. Presented at the fourth annual meeting of the Canadian Phytopathological Society held at Macdonald College, P.Q., June 26 and 27, 1934.

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attributed by him to be due, in part at least, to the operation of the factor of biological antagonism. Although the importance of this factor in diseases caused by soil-borne fungi can not be over-looked, it would seem, however, that, in attempting to explain the numerous contradictions in the earlier work, consideration must be given to the differences in experimental procedure and the method of recording disease data used by different workers.

Previous experience with numerous pot-culture experiments emphasized the importance, if reliable and reproducible results are to be expected, of controlling in a satisfactory manner the conditions making up the soil environment and of standardizing as far as possible the method of recording the degree of infection caused by cereal root-rot fungi. In other words, experience has led to the adoption of more scientific methods in conducting greenhouse experiments. The methods used in pot tests with cereal root-rot fungi are given in considerable detail in this paper.

Type and Treatment of Soil

The importance of soil type in experiments with soil-borne pathogens is demonstrated by the studies of Garrett (*9*), Henry (*11*), and Moritz (*17*). In the present study a uniform mixture of soil consisting of $\frac{2}{3}$ fine clay loam and $\frac{1}{3}$ sand was used. The soil was placed in 6-inch flower pots and steam-sterilized for 4 hours at 15 pounds pressure. Immediately after sterilization the fungus to be tested was added to the soil. The subsequent treatment of the soil was such as to prevent its reverting to the non-sterile condition.

Kind of Inoculum and Method of Infesting Soil

Inocula were prepared by growing the fungi on a finely-ground, oat-hull medium. This medium, when completely over-grown with sporulating mycelium of the fungus, was thoroughly mixed with the sterile soil, one part of inoculum to 100 parts of soil by weight. All soil for a given experiment was prepared at the same time.

Soil Temperature and Moisture

At the commencement of an experiment each pot of soil was adjusted to 55% of its total moisture-holding capacity and weighed. This moisture content was maintained by weighing the pots at 2-day intervals and adding sterile water to restore to original weight. A greenhouse furnished with temperature-control apparatus was used for the experiments. During the course of any given trial the soil temperature was controlled within the range 22° to 25° C., while the range of air temperature was from 20° to 28° C.

Seed and Seed Treatment

Five varieties of wheat were used in the present study: Marquis, Mindum, Marquis × Pentad Strain 729, Marquis × Pentad Strain 726 and H–44–24 × Reward Strain 716. The seed was obtained from pure line material and hand-selected for size and plumpness. Each lot of seed was surface-sterilized by rinsing in 80% alcohol, then soaking in a solution of mercuric chloride (1 : 1000) for 3 minutes, rinsing again in 80% alcohol and afterwards thoroughly washing in sterile water. The results of adequate germination tests established that from 96 to 100% of the surface-sterilized seeds were viable.

The seed was sown immediately after sterilization. By means of a small sterile tube 25 holes were made uniformly at a depth of one inch

below the surface in each pot of soil. A single seed was planted in each hole and covered by a light packing of the surface layer. The soil was only slightly disturbed, and the possibility of foreign organisms entering the experimental soils was markedly reduced by this procedure. In order to allow the fungi to become well established in the soil to which they were added, three days were allowed to elapse before planting of the seed.

Recording the Amount of Disease

Accurate measurement of the intensity of infection is essential in studying cereal root-rot fungi. If the results are to be reliable and reproducible the amount of disease must be accurately recorded in a convenient and simple form. The work of Jones, McKinney and Fellows (*13*) on potato scab, and of McKinney (*16*) on a *Helminthosporium* disease of wheat, has demonstrated that it is not sufficiently adequate to use alone either the number of individual plants infected or the degree of infection as the index of the amount of disease. McKinney (*16*) expressed the extent of disease caused by *H. sativum* as an infection rating which took into account the percentage of the total number of plants infected as well as the degree of infection. A modification of McKinney's method seemed well adapted for the investigation of factors influencing the pathogenicity of cereal root-rot fungi, and is used in the present study. The infection classes with their respective numerical ratings are shown in Figure 1.

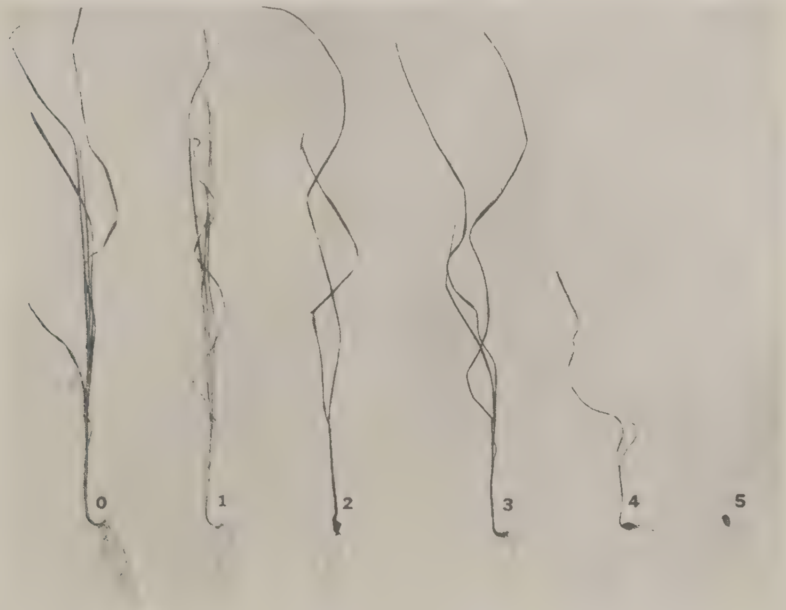

FIGURE 1—Reward wheat seedlings showing infection classes and numerical ratings used in measuring the amount of infection caused by *Helminthosporium sativum* in pot tests. Classes: (0) Healthy, no infection. (1) Small lesions on roots and base of culm. (2) Distinct lesions on base of culm, sheath and roots. (3) Base of culm and root system rotted and markedly reduced. (4) Severe rotting of roots and basal part of plant, plant severely stunted. (5) Seed fails to germinate or plumule and root system rotted or completely destroyed.

In pot tests with cereal root-rot fungi it was considered that any other index of disease intensity, to be of value, must bear relation to the disease rating. In order to determine suitable criteria for measuring the intensity of root rot of wheat caused by species of *Helminthosporium* and *Fusarium* a statistical study was made of the data from properly controlled pot tests. The results clearly established that there was a significant negative correlation between the amount of disease, as expressed by the disease rating, and the total dry weight of plants, while no such association existed between total length of plants and disease rating. In the present study the dry weight of plants was used as an index in determining the comparative effects of *C. roseum* on the pathogenicity of *H. sativum*.

Analysis of Data

The results of previous pot-culture studies on factors influencing the pathogenicity of cereal root-rot fungi have demonstrated that differences in pathogenicity may easily be of small magnitude and require data of a relatively high order of precision for their establishment. Consequently, in order to insure that the observed differences indicated, within the limits of experimental error, the effects of the applied treatments or organisms, the experiment was properly arranged and adequately replicated. The disease rating and dry weight data were treated statistically by the "Analysis of Variance" and "*Z*" test. It is not possible here to illustrate these methods in detail and reference should be made to Fisher (*6*), Fisher and Wishart (*7*) and Goulden (*10*).

THE PATHOGENCITY OF *HELMINTHOSPORIUM SATIVUM* AS INFLUENCED BY *CEPHALOTHECIUM ROSEUM*

In earlier preliminary studies on root-rot and foot-rot diseases of cereals in Manitoba, *Cephalothecium roseum* Corda was frequently isolated from cereal plants grown in soil artificially infested with species of *Fusarium* and *Helminthosporium*. It was observed that the virulence of the root-rot pathogens was markedly reduced in the presence of *C. roseum*. These results suggested further experiments to determine the real effect of *C. roseum* on the virulence of *H. sativum*.

Pot-culture tests were made to study the pathogenicity of *H. sativum* alone, and in association with *C. roseum*. The methods of soil management and infestation, planting of the seed, harvesting of the plants, and recording and analyzing the experimental data used have already been described in the previous section.

The particular pathogen investigation was a fast-growing strain of *Helminthosporium sativum* P., K., and B. which was originally isolated in 1932 from diseased roots of wheat. The pathogenicity of this fungus on wheat, oats, barley and rye was established by numerous greenhouse pot tests. The strain of *Cephalothecium roseum* used was isolated in 1933 from a surface sample of Manitoba soil.

Wheat plants were grown in each of the following types of soil:—

(1) Sterile soil infested with *H. sativum*.

(2) Sterile soil infested with *C. roseum*.

(3) Sterile soil infested with *H. sativum* and *C. roseum*—the amount of oat-hull inoculum of each fungus being one-half that used in (1) and (2).

(4) Sterile soil infested with *H. sativum* and *C. roseum*—the amount of inoculum of each fungus being the same as that used in (1) and (2).

(5) Sterile soil plus oat-hull medium (Control B).

(6) Sterile soil alone (Control A).

The pathogenic capabilities of the fungi separately, and in association, were determined on five varieties of wheat. One hundred surface-sterilized seeds of each variety were planted in four pots of each type of infested and uninfested soil. Thus there were 20 pots of each soil type, the complete experiment consisting of 120 pots. In order to take care of place effect the pots were properly randomized on the greenhouse bench. All soil was prepared, sterilized and infested, and the seed planted at the same time, so that all conditions, with the exception of the soil fungi, were comparable. Uniform conditions of soil moisture and temperature were maintained during the period of the experiment. The experiment was replicated three times.

Germination counts were made 15 days after planting. It was observed that the germination of seed of all wheat varieties was markedly reduced in pots of sterile soil to which *H. sativum* had been added, while the presence of *C. roseum* alone in sterile soil did not affect germination. The germination results with sterile soil, sterile soil plus oat-hull medium, and sterile soil infested with *C. roseum* were almost identical. It was observed, however, that the detrimental effect of *H. sativum* on seed germination was decidedly reduced in the presence of *C. roseum*, the percentage of germination being considerably higher in soil where *H. sativum* was associated with *C. roseum*.

At the end of the experimental period (36 days) the plants were washed free of soil, examined, and the degree of root-rot infection was recorded according to the methods described above. The plants were air-dried and the total dry weight of each variety in each soil series was obtained. A general examination of the disease rating and dry weight results indicates a real difference in the pathogenicity of *H. sativum* on wheat. In order to verify the results a thorough examination of the data was made. Table 1 gives the results of the complete analysis of variance for disease rating and total dry weight of plants.

The significance of the results is assessed by the *Z* test (Fisher (*6*)), in which the variance due to any known cause is compared with the variance due to error. The values of *Z* in Table 1 greatly exceed the 1%

TABLE 1.—ANALYSIS OF VARIANCE: DISEASE RATING AND DRY WEIGHT OF PLANTS

Disease rating

	Degrees of freedom	Sum of squares	Mean square	Z	1 per cent point
Experiments	3	1580.54	526.85		
Varieties	4	1403.11	350.78	0.5594	0.6325[1]
Soil treatments	5	134655.54	26931.11	2.7183	0.5871
Soil treatments × varieties	20	1371.60	65.58		
Error	87	9970.56	114.60		
Total	119	148981.35			

TABLE 1.—ANALYSIS OF VARIANCE DISEASE RATING AND DRY WEIGHT OF PLANTS—*Concluded*

Dry weight of plants

	Degrees of freedom	Sum of squares	Mean square	Z	1 per cent point
Experiments	1	12.24	12.24		
Varieties	4	46.13	11.53	1.2980	0.6987
Soil treatments	5	101.40	20.28	1.5803	0.6576
Soil treatments × varieties	20	6.22	0.31		
Error	29	25.06	0.86		
Total	59	191.05			

[1] 5% significant level required for $n_1=4$, $n_2=87=0.4535$.

points and indicate that a high degree of significance can be attached to the differences between the pathogenicity of *H. sativum* in the various soil series. Differences between varieties of wheat are also significant. With the establishment of significant differences in pathogenicity and varieties, a detailed examination of the results was made. The standard errors of the means of soil treatments and varieties are given in Table 2. This table presents a complete summary of the results of the experiment.

TABLE 2.—SUMMARY OF THE RESULTS OF THE EFFECT OF *Cephalothecium roseum* ON THE PATHOGENICITY OF *Helminthosporium sativum* ON WHEAT SEEDLINGS (AVERAGE OF FOUR TRIALS)

Disease rating

Organisms added to sterile soil	Amount of oat-hull inoculum per pot of sterile soil	Variety					Mean of soil treatments	Standard error of means
		Mindum	Strain 729	Strain 726	Marquis	Strain 716		
	grms.							
Control A	0	6.8	3.1	9.0	16.5	9.3	8.9	
Control B [1]	0	13.7	9.7	15.0	15.1	13.4	13.4	
C. roseum	100	5.9	9.8	4.0	20.0	12.0	10.3	2.39
H. sativum	100	81.3	84.5	86.1	82.2	91.3	85.1	
C. roseum + *H. sativum*	50 + 50	68.6	68.8	80.1	76.9	79.9	74.9	
C. roseum + *H. sativum*	100 + 100	61.7	67.7	75.6	74.6	81.1	72.1	
Mean of varieties		39.7	40.6	45.0	47.5	47.8	44.1	
Standard error of means		2.18						

Total dry weight of plants (grams)

Control A	0	6.3	5.3	4.6	3.3	4.0	4.7	
Control B [1]	0	4.9	2.8	2.3	2.4	2.7	3.0	
C. roseum	100	5.4	3.7	3.8	2.6	3.9	3.9	0.29
H. sativum	100	1.6	0.9	0.9	0.4	0.7	0.9	
C. roseum + *H. sativum*	50 + 50	3.4	1.8	0.9	0.6	1.4	1.6	
C. roseum + *H. sativum*	100 + 100	4.2	2.3	1.4	0.9	1.8	2.1	
Mean of varieties		4.3	2.8	2.3	1.7	2.4	2.7	
Standard error of means		0.27						

[1] One hundred grams of sterile oat-hull medium added per pot of sterile soil.

Examination of the disease-rating section of Table 2 shows that the strain of *H. sativum* used in this investigation was distinctly pathogenic on wheat, while the fungus *C. roseum* was decidedly non-pathogenic. The important point in the experiment is that the presence of *C. roseum* suppressed to a significant degree the virulence of *H. sativum*. The comparative effect of these fungi alone, and in association, on wheat seedlings, is shown in Figure 2.

FIGURE 2—The pathogenicity of *Helminthosporium sativum* as influenced by *Cepholothecium roseum* in pot tests. Growth of wheat plants at 36 days. (1) Marquis, (2) Mindum, and (3) H–44–24 × Reward Strain 716. Arrangement of soil series: (A) Control, no fungus, sterile soil plus 100 grams of sterile oat-hull medium. (B) *C. roseum* in sterile soil, 100 grams of oat-hull inoculum. (C) *H. sativum* in sterile soil, 100 grams of oat-hull inoculum. (D) *H. sativum* and *C. roseum* in sterile soil, 50 grams of oat-hull inoculumof each fungus. (E) *H. sativum* and *C. roseum* in sterile soil, 100 grams of oat-hull inoculum of each fungus.

From the results in Table 2 it is evident that the antagonism of *C. roseum* to *H. sativum* was not significantly influenced when the amount of inoculum of each fungus added to the soil was increased from 50 to 100 grms. per pot. The results of the experiment indicate the importance of the reaction of one living micro-organism upon another in the soil, and confirm the experiments of Henry (*11*), Sanford and Broadfoot (*19*) and Garrett (*9*), who found that infection of wheat seedlings by root-rot fungi was suppressed by the antagonistic effect of certain fungi and bacteria.

It may be mentioned in passing that the analysis of the results shows that, of the varieties used in this study, Mindum was the most resistant while Marquis and H–44–24 × Reward Strain 716 were the most susceptible to attack by the strain of *H. sativum* used in the experiment.

It is difficult, even under the most carefully controlled conditions, to prevent inocula of saprophytic fungi from entering pots of sterile soil. Henry (*11*) found that the addition of a trace of unsterilized soil to pots of sterile soil served as a source of saprophytic soil organisms which subsequently produced a marked inhibitive action on the development of cereal root-rot fungi.

The results in Table 2 show significant differences between dry weight of plants grown in sterile soil and in sterile soil plus the oat-hull medium, and suggest that the addition of organic material to sterile soil stimulates the development of saprophytic organisms, thereby producing a condition of the soil environment which is unfavourable for the growth of wheat seedlings. The results seem to indicate that the competition for food among soil organisms and the staling products produced by these organisms are important factors influencing plant growth. The various inter-relationships between the soil organisms themselves and between these organisms and the plants, as well as the influence of the environment on these inter-relationships are imperfectly understood. This phase of the root-rot problem is exceedingly complex and requires further investigation.

While the results of the foregoing experiment indicate the importance of the factor of biological antagonism in the control of root-rot diseases of cereals, further studies are needed to determine the real nature of the antagonism involved.

The ability of *C. roseum* to parasitize other fungi has been reported by Whetzel (*22*) and Boning (*3*). During the course of an intensive investigation of the black knot disease of plums and cherries (*Dibotryon morbosum* (Sch.) T. and S.) Koch (*14*) found that in nature *C. roseum* actively parasitizes the stroma of *D. morbosum*, and exerts an important measure of biological control over this fungus. Sanford and Broadfoot (*19*) studied the effects of certain soil-inhabiting micro-organisms on the virulence of *Ophiobolus graminis* Sacc. and found that staling products produced by some fungi were effective in suppressing the pathogenicity of *O. graminis* in soil culture. Vasudeva (*20*), Machacek (*15*), and Sanford (*18*) concluded that the pathogenicity of certain fungi was suppressed by the staling products produced by other fungi and bacteria.

Preliminary tests were made to determine what effect staling products produced by *C. roseum* and certain other fungi on various liquid media (staled solutions) had on the germination and germ-tube growth of spores of *H. sativum*. These tests comprise a part of the physiological studies under investigation at this laboratory, the complete results of which will appear in a subsequent paper.

The results presented in Table 3 show that the germination of spores and growth of germ tubes of *H. sativum* were markedly suppressed when they were cultured in staled solutions of other fungi, particularly *Penicillium expansum* Link, *Aspergillus niger* Van Tiegh. and *Cephalothecium roseum*. The results with *C. roseum* support the view that toxic substances produced by this fungus when grown in soil were the chief factor in suppressing the virulence of *H. sativum*.

TABLE 3.—EFFECT OF STALED SOLUTIONS OF VARIOUS FUNGI ON THE GERMINATION AND GERM-TUBE GROWTH OF SPORES OF *Helminthosporium sativum* (AVERAGE FOUR TRIALS)

Germination rating of spores of *Helminthosporium sativum*[1]

Organism	Soil extract	Modified Duggar's medium	Waksman's Sucrose Peptone solution	Mean of organism
Penicillium expansum	0.0	3.5	14.6	6.0
Aspergillus niger	0.0	0.0	48.5	16.2
Cephalothecium roseum	9.8	23.7	26.7	20.1
Helminthosporium sativum	9.6	2.9	52.9	21.8
Trichoderma koningi	75.2	24.5	21.9	40.5
Fusarium culmorum	46.1	29.8	62.5	48.8
Rhizoctonia Solani	51.3	77.0	74.0	67.4
Control (no organism)	99.5	77.7	100.0	92.4

[1] Germination rating based on the percentage of spores germinated and length of germ tubes.

$$\text{Germination rating} = \frac{\text{Sum of numerical ratings of individual spores} \times 100}{\text{Total number of spores examined} \times 5 \text{ (Highest numerical rating)}}$$

The results reported in this paper were obtained under a definite set of environmental conditions and are undoubtedly much more pronounced than might be expected under field conditions. It is well known, however, that the soil supports a varied and large microflora, and that soil temperature and moisture markedly influence the biological balance in the soil. There is little doubt but that, to some extent at least, the presence of *C. roseum* in the soil exerts an inhibiting effect on the pathogenicity of *H. sativum*. The results of this study confirm the work of Henry (*11*), Sanford and Broadfoot (*19*), and Garrett (*8*, *9*), and suggest that biological antagonism may be one of the most important factors influencing the extent of damage caused by cereal root-rot fungi in the field.

SUMMARY

Greenhouse experiments have been made to determine the influence of the saprophytic fungus *Cephalothecium roseum* Corda on the pathogenicity of *Helminthosporium sativum* P., K., and B. in the soil. A detailed discussion of the methods and analysis employed in pot-culture experiments with cereal root-rot fungi are given.

A fast-growing strain of *H. sativum* was distinctly pathogenic on wheat. The pathogenicity of this strain was suppressed by the antagonistic action of *C. roseum*.

Preliminary physiological studies have shown that staling products produced by *C. roseum* on liquid media were exceedingly effective in inhibiting the germination and germ-tube growth of spores of *H. sativum*. The results suggest that the toxicity of substances produced by *C. roseum* was the chief factor in suppressing the pathogenicity of *H. sativum*.

Pot-culture experiments have demonstrated that *C. roseum* exerts a measure of natural biological control over *H. sativum*.

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Résumé

Etudes sur le traitement de la pourriture de la racine des céréales causée par *Fusarium culmorum* (W.G. SM.) Sacc. et *Helminthosporium sativum* P.K. et B. II. Pathogénicité de *Helminthosporium sativum* influencé par *Cephalothecium roseum* Corda dans les essais de pots en serre. F. J. Greaney et J. E. Machacek, Laboratoire fédéral de recherches sur la rouille, Winnipeg, Man.

Il a été fait des essais en serre pour déterminer l'influence du champignon saprophytique *Cephalothecium roseum* Corda sur la pathogénicité de *Helminthosporium sativum* P.K. et B. dans le sol. Une étude détaillée des méthodes et des analyses employées dans les essais de culture en pots des champignons de la pourriture de la racine des céréales est donnée. Une espèce à pousse rapide de *H. sativum* s'est montrée tout à fait pathogène sur le blé. La pathogénicité de cette espèce a été supprimée par l'action antagonistique de *C. roseum*. Il a été démontré par des études physiologiques préliminaires que les produits de *C. roseum* sur le milieu liquide étaient extrêmement utiles pour enrayer la germination et la végétation des tubes de germes des spores de *H. sativum*. Les résultats indiquent que la toxicité des substances produites par *C. roseum* est le facteur principal dans la suppression de la pathogénicité de *H. sativum*. Il a été démontré par des essais de culture en pots que le *C. roseum* exerce un certain contrôle biologique naturel sur le *H. sativum*.

OCCURRENCE OF "SPOTTED WILT" OF TOMATO IN ONTARIO[1]

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In 1931 the Dominion Botanist brought to the writer's attention while at Ottawa, a diseased condition of tomatoes which, though of the streak type, did not in all respects agree therewith. Specimens were sent to St. Catharines the following year but pressure of other work made it impossible to pay much attention to this disease until 1934, when fresh specimens were obtained and the disease studied.

Symptoms of the Disease at St. Catharines

At St. Catharines, under greenhouse conditions, the disease has shown symptoms that strongly suggest the trouble to be spotted wilt, as described by Samuel, Bald and Pittman (*8*) and K. Smith (*10*). The outstanding symptoms are bronzing (Plate I, 1) of young leaves on rapidly growing plants, followed by a pronounced stunting of the plant (Plate I, 2). The bronzing may take the form of spots, or may be fairly generally distributed over the leaf surface and may vary in intensity from a very inconspicuous glaze to a distinct dark brown to almost black glazed area. Generally bronzed leaves turn downward and inward, and bronzed areas often become necrotic and dry out. An additional symptom that is of some value from a diagnostic standpoint is that the leaves at the top of affected plants curl lengthwise and inwards to a very pronounced degree, giving a leaf roll appearance to the top leaves (Plate I, 3). At least this symptom has been quite constant under glasshouse conditions at St. Catharines.

The rapidity with which symptoms appear and change is remarkable. A plant at night that shows faint bronzing may the next morning exhibit definite and pronounced bronzing and prominent zoned rings may form within a few days' time. In addition to the bronzing, certain leaves and particularly certain plants have shown a profusion of brown concentric rings, or a pattern network of concentric lines, on the leaf tissue (Plate III, 4, 5 and 6). On the stalk of one old plant, and on the petioles of several young plants superficial necrotic streaks have been observed, but such necrotic streaks seem to be rare with this disease.

There are in the greenhouse at time of writing three plants which three weeks ago exhibited pronounced bronzing and cessation of growth, with no necrosis. These plants have in the meantime resumed growth and the symptoms on the secondary growth are not as pronounced as they were shortly after the plants had become infected. Though bronzing is still present, it is quite inconspicuous and might easily be overlooked. Also necrosis of tissue which was absent in the early stages of the disease is now present though not severe. In fact a somewhat superficial inspection

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of these plants might well result in a diagnosis of "streak" but a more careful examination would show that bronzing was present, and that much of what at first might be taken for necrosis is actually "black bronzing" which from above looks something like "necrosis."

All these symptoms as observed at St. Catharines are in such close agreement with those described for spotted wilt by Samuel, Bald and Pittman (*8*) that there would appear to be little room for doubt but that this disease, or one closely related to it, is now present in Ontario. So far, however, this disease has been studied under glasshouse conditions only, and therefore additions to the symptomatology may have to be made after it has also been studied under field conditions.

Symptoms of Spotted Wilt Distinct from Tomato Streak

It might be well at this time to point out that the symptoms of spotted wilt are quite distinct from streak. Generally the first symptom of streak is a black necrosis of the leaf or stem tissue, with or without accompanying mosaic mottling. This necrosis generally progresses rapidly until the outstanding symptom of streak is black necrosis, particularly of stem, petiole and leaf, though the fruit also is often affected with raised, black, irregularly-shaped areas. In the case of spotted wilt, however, the initial symptom on tomato is a bronzing of the leaf surface with or without "rings" and various patterns made up of "concentric lines." No necrosis of leaf or stem tissue is present at first, but later some necrosis may occur (Plate I, 1), though it is generally absent, and, if present, is but slight in extent compared with streak. On the fruit also the difference in symptoms is pronounced as spotted wilt generally occurs as bronze or yellow blotches often in the form of almost perfect concentric circles.

Literature

Spotted wilt was first found near Melbourne, Australia, by Brittlebank (*3*) in 1919. A more complete description of the disease, including the insect vectors, was published in 1930 by Samuel, Bald and Pittman (*8*) and in 1931 by Bald and Samuel (*1*). In 1931 K. Smith (*11*) reported this same disease as occurring in the British Isles. In his second paper Smith (*10*) describes the symptoms, insect vectors and host range in considerable detail. In 1929 Beecher and Shapovalov (*2*) and later Shapovalov (*9*) report a tomato die-back which they say is "symptomatologically similar to spotted wilt." In 1930 Doolittle and Sumner (*5*) described a diseased condition of tomatoes as found in Wisconsin which they considered to be spotted wilt. Later in 1934 (*6*) they published the evidence on which their previous report was made. In 1932 Conners (*4*) reported spotted wilt in the Canadian Plant Disease Survey for that year, as having been found in Saskatchewan. In 1934 McWhorter (*7*) reported that the English form of spotted wilt occurred in Oregon.

Distribution

The distribution of spotted wilt is as follows: Australia—Victoria, New South Wales, South Australia, West Australia, Queensland and Tasmania; British Isles; United States of America—Oregon, Wisconsin and California; and Canada—Ontario and Saskatchewan.

Plate I.—1. Leaves of Grand Rapids variety showing characteristic bronzing. This bronzing appears as a brown, glazed area. Necrosis is apparent towards tip of right-hand leaf. 2. Healthy tomato plant (left) and spotted wilt plant (right). Note stunting of diseased plant and curling inwards and downwards of top leaves. 3. The tips of two tomato plants, healthy (above) and spotted wilt (below). Note 'leaf rolling' of diseased plant.

PLATE II.—1. Spotted wilt on tomato leaves of Grand Rapids variety. Note 'rings' and 'spots' characteristic of this disease. 2. Spotted wilt on *Nicotiana glutinosa* (left), and tomato streak (single virus) on *Nicotiana glutinosa* (right). Note that the local necrotic lesions produced by spotted wilt virus are much larger than those produced by tomato streak virus.

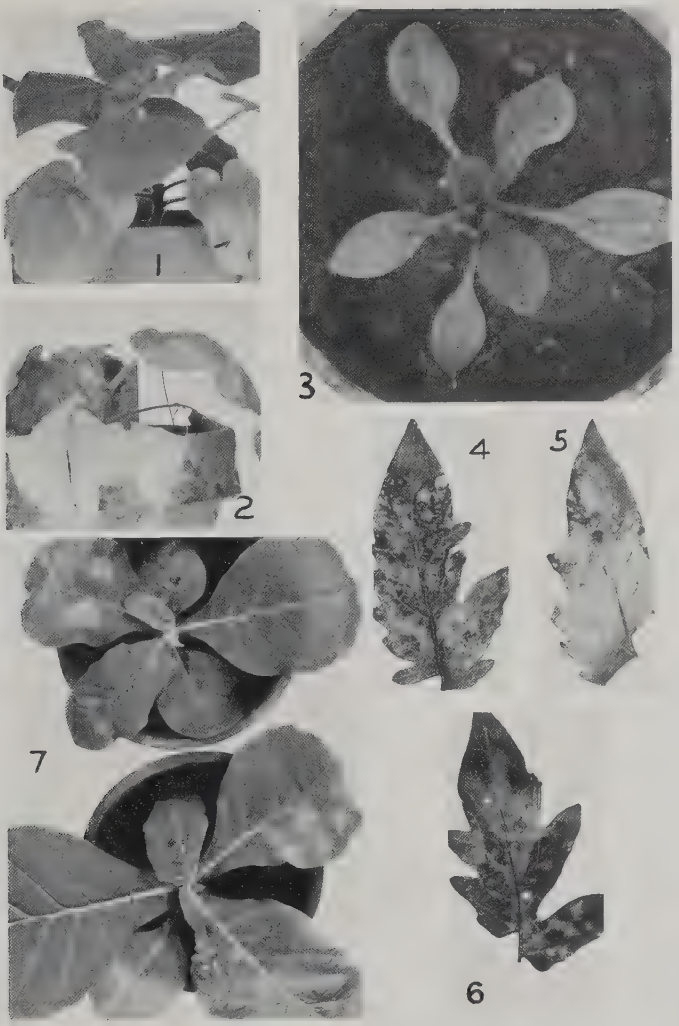

Plate III.—1. Spotted wilt virus in *Nicotiana glutinosa*. Note local necrosis at arrow and systemtic necrosis in tip of plant. 2. Later stage of spotted wilt on *N. glutinosa*. Plant wilted and almost dead. 3. Spotted wilt on Petunia. Note local necrotic lesions which showed up three days after inoculation and were photographed ten days after inoculation. 4, 5, and 6. Spotted wilt on tomato, showing concentric rings and 'patterns' of concentric lines. 7. Spotted wilt on tobacco (Burley, Standup Resistant) showing large local necrotic lesions and systemic necrosis.

Importance of Spotted Wilt

In Australia and England where this disease has been under investigation for some years spotted wilt is considered to be a very serious disease. In this connection Brittlebank (Australia) (*3*) writes "Spotted wilt was first observed during the 1915-16 season, when the injury was slight owing to the restricted area over which the disease had spread, and to the comparatively small number of plants affected. In the following year the number of diseased plants had increased to an alarming extent. The present season (1918–19) has, to say the least, been disastrous, and fully 50 per cent of suburban garden plants have been destroyed. In the county districts the disease has made considerable progress and diseased plants have been found in nearly all parts of Victoria." K. Smith (*10*) England, writes "In the writer's opinion spotted wilt is the cause of considerable loss to the tomato growing industry in the British Isles . . . ." Also as K. Smith points out, spotted wilt is apt to be more serious in a glasshouse where plants of various kinds are propagated, since in such a house the chances of "over wintering" are greatly increased, due to the fact that the spotted wilt virus attacks not only the tomato but many other plants as well, such as primula, gloxinia, cineraria, dahlia, winter cherry, chrysanthemum, aster, zinnia, petunia, lupine, etc. Any one of these plants may well act as a "carrier" in "over wintering" the virus from one host to another.

Just how spotted wilt will behave in Canada has yet to be ascertained. The climate in Canada is so varied and dissimilar to that in either Australia or England that the question of possible importance in Canada cannot be answered at this time.

At the present time its known distribution in Canada is confined to Ontario and Saskatchewan. However, as there is every possibility that this disease may be serious in Canada if it becomes widely distributed, every effort should be made to ascertain its present distribution. It is with this idea in mind that this preliminary paper is published so as to establish the fact of its presence in Ontario in order that plant pathologists and growers alike may be on the look-out for it.

Experimental

Inoculations were effected by mechanical sap transfer by means of gentle rubbing with a pad of absorbent cotton moistened with the virus. It was found that the percentage of successful inoculations was comparatively low since in several tests only two out of six plants became infected. In a few tests where young vigorously-growing tomato plants were inoculated with virus obtained from a recently-infected plant, 100% positive results were obtained.

In all, twenty *Nicotiana glutinosa*, twenty *Nicotiana tabacum* (tobacco, Standup Resistant), twenty-five Petunia (garden variety) and fifty *Solanum lycopersicum* (tomato, Grand Rapids) have been inoculated, and characteristic symptoms obtained on all hosts.

In the first series of inoculations four plants each of tomato, tobacco and *N. glutinosa* were inoculated and systemic necrosis occurred on one

tobacco plant only. All the other inoculations gave negative results. Sub-transfers were made from this necrotic tobacco plant to two plants each of tomato, tobacco and *N. glutinosa*, with negative results in every case. Later another series was inoculated with inoculum from the same plant, and positive results were obtained on all tomato and *N. glutinosa* plants, but none on tobacco.

In another series six plants were inoculated and positive results were obtained on one *N. glutinosa*, one tobacco and two tomato plants. A later series of inoculations gave 100% positive results on tobacco and *N. glutinosa* but only 50% on tomato.

As has been recorded by Smith (*10*) the petunia is a very suitable host to use as indicator as it produces local lesions within two days' time. At St. Catharines the garden variety of petunia has given local lesions in from two to three days' time, another point which suggests that the virus under investigation here is similar to the English spotted wilt virus.

As a result of successful inoculations on four different hosts, the following symptoms were obtained:—

Tomato: The symptoms have already been outlined above, but bronzing and severe stunting of growth in young plants, and the curling lengthwise and inwards of younger leaves may be mentioned here (Plate I, 1, 2 and 3; Plate III, 4, 5 and 6).

Tobacco: Generally the first symptom takes the form of large local lesions on inoculated leaves followed by severe systemic necrosis and death of plant (Plate III, 7).

Nicotiana glutinosa: Here also the first symptom takes the form of large local lesions followed by systemic necrosis (Plate II, 2; Plate III, 1 and 2). In some cases no local lesions developed, though systemic necrosis resulted in death of plant.

Petunia: Within three days from time of inoculation, circular brown local lesions (Plate III, 3) occur on the rubbed leaves. Later the centre of these spots becomes yellowish, while the margin remains black.

The viruses of "spotted wilt" and "single virus streak" both produce local lesions on *Nicotiana glutinosa* and *N. tabacum*. A comparison of the local lesions produced by these two viruses points out (*1*) that the spotted wilt virus produces a much larger lesion on both *N. glutinosa* and *N. tabacum* than does the single streak virus, and (2) that on *N. glutinosa* the local lesion produced by the single streak virus has a much more pronounced outer ring of dark brown colour than does the local lesion produced by spotted wilt virus.

On *N. tabacum* the spotted wilt virus produces lesions with a diameter of 5 to 7 mms. six days after inoculation, whereas the single streak virus produces lesions with a diameter of only 1 to 2 mms. in the same time.

On *N. glutinosa* the spotted wilt virus produces lesions with a diameter of 2 to 3 mms., whereas the single streak virus produces lesions of only 1 mm. in diameter in the same time (Plate II, 2 and 3).

Longevity of the Virus

According to Bald and Samuel (*1*), and Smith (*10*) who have studied spotted wilt in Australia and England respectively, the potency of the virus lasts for only four to five hours after it has been extracted from the plant. Both investigators agree that after six hours the virus has lost its potency.

A single test made at St. Catharines would indicate that the virus under investigation here has also a very short period of potency.

TABLE 1.—EFFECT OF STANDING ON VIRUS

Number of plants inoculated	Number of plants infected after			
	Fresh	½ hour	2 hours	6 hours
3 petunia in each case	3	2	–	–

This test would indicate that the virus being investigated at St. Catharines loses its potency somewhere between ½ hour to 2 hours after standing at room temperature.

Resistance to Heat

Australian spotted wilt is inactivated by heat at very low temperatures. Bald and Samuel (*1*) give the thermal death-point as in the neighbourhood of 42° C., for ten minutes heating. A preliminary test at St. Catharines has shown that the virus under observation here is inactivated by heating at 45° C., for ten minutes.

These preliminary results present good evidence for considering that spotted wilt is now present in Ontario.

Suggested Control Measures

Spotted wilt is a "virus" disease that is known to be spread by *Thrips tabaci* Lind., and Thrips *Frankliniella insularis*. Also since the disease can be spread artificially by juice transfer it is quite likely that spread may take place in a greenhouse during pruning and stopping operations.

Therefore if this disease is found in a greenhouse, or field, it is advisable to destroy all diseased plants so as to cut down the possibility of spread. In the greenhouse, thrips should be kept down to a minimum.

In case of doubt as to diagnosis it is suggested that *fresh* specimens of suspected plants be sent to the St. Catharines laboratory for diagnostic purposes. As the virus of this disease lives only a short time in plant parts when excised, it is suggested that specimens sent in include, when possible, green fruits. The virus apparently exists longer in green fruits than in other parts of the plant.

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Résumé

La brûlure en taches des tomates fait son apparition dans l'Ontario. G. H. Berkeley, Laboratoire fédéral de pathologie végétale, St. Catharines, Ont.

Il a été démontré par des essais d'inoculation que la maladie à virus des tomates connue sous le nom de "Brûlure en taches" (*Spotted Wilt*) sévit actuellement dans l'Ontario, au Canada. L'auteur décrit les symptômes de la maladie sur la tomate, le tabac, le pétunia et le *Nicotiana glutinosa* et il indique la longévité du virus (2 heures) et sa résistance à la chaleur (45° C.). La liste des hôtes connus du virus comprend 13 plantes. Les moyens répressifs recommandés consistent à arracher les plantes malades et à réduire au minimum le nombre de *Thrips*.

THE PHYSIOLOGY OF *RHIZOCTONIA SOLANI* KÜHN[1]

III. THE SUSCEPTIBILITY OF DIFFERENT PLANTS AS DETERMINED BY SEEDLING INFECTION

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The object of determining the susceptibility of different seedlings to infection by *Rhizocotonia Solani* Kuhn, was to find resistant crops suitable for planting in rotation upon Rhizoctonia infested soils, to aid in the control of this fungus. This fungus is known to attack over 160 species of plants (*3*) including field, vegetable and ornamental crops and weeds (*5*). Not only is it a destructive parasite on many crops but is also a saprophyte capable of existing indefinitely on dead organic matter in the soil (*5*). However, it has been found that the amount of the fungus in the soil is usually great before serious infection takes place (*5*, *8*). It is probable that the planting of susceptible crops will aid in the maintenance and increase of this fungus in the soil. On the other hand the planting of resistant crops may tend to reduce the infestation in soils. This possibility is suggested by the work of Chamberlain (*1*) who found a reduction in soil infection when potatoes followed grass but not when following cereals, peas or turnips.

Although, as mentioned above, over 160 species of plants have been found to be susceptible to *Rhizocotonia*, much evidence of varietal resistance has been observed and recorded. Zeller (*11*) working on black root of strawberries found that Clark's Seedling, Marshall and Magoon are susceptible, while Ettersburg 121 and the wild coast strawberry (*Fragaria chiloensis* Duchesne) are either immune or highly resistant. Tilford (*10*) observed that Kentucky Blue Grass (*Poa pratensis* L.), Crab grass (*Panicium sanguinale* L.), Bermuda grass (*Cynodon dactylon* L.), and white clover (*Trifolium repens* L.), are immune while red fescue (*Festuca rubra* L.) and some of the bent grasses (*Agrostis* spp.) are susceptible. Teng (*9*) working on *Rhizoctonia* of lobelia found that *L. erinus* L. var. *gracilis*, was resistant to infection but *L. erinus* L. var. *speciosa* and *L. ramosa* L. succumbed to infection when the roots were wounded and under the same conditions. *L. cardinalis* L. was markedly stunted.

EXPERIMENTAL

Two types of experiments were carried out. In the first the seedlings were grown on a nutrient agar,[3] in Petri dishes previously inoculated with a strain of the fungus isolated by germinating the sclerotia from a Netted Gem potato grown at the Experimental Station, Saanichton, B.C. The seed was first surface sterilized by immersion in a 1 : 1000 solution of mercuric chloride. This treatment was followed by a rinse and a one-hour soak in sterile tap water. Smooth seeds such as peas and beans were

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[3] The formula found to be most satisfactory for seedling and fungus development was made up as follows:

Potassium nitrate	10 g	Sucrose	20 g
Potassium di hydrogen phosphate	5 g.	Agar	15 g
Magnesium sulphate	2 5 g.	Water	1000. cc.
Iron chloride	trace		

TABLE 1.—SHOWING THE PATHOGENICITY OF *Rhizoctonia Solani* KUHN TO DIFFERENT PLANT

A. As determined by the lesions on the roots when the seedlings were grown on nutrient agar inoculated with the fungus.
B. As determined by the lesions on the roots when the seedlings were grown on inoculated soil.
C. As determined by the growth of the plants.

Species	Variety	A	B	C
Vetch	Purple	10	—	—*
	Oregon	8	2	2
	Hungarian	8	—	—
Pea	Champlain	8	—	—
	Capital	8	3	3
	Blue Bell	5	2	2
Bean	Yellow Eye	10	3	2
	Ede's Pole	9	3	2
Alfalfa	Grimm	10	0	1
	Cossack	10	—	—
Clover	Chateaugay—Red	10	0	0
	Early Swedish—Red	6	0	2
	Late Swedish—Red	10	—	—
	Crimson	10	0	0
	Mammoth White Dutch	10	0	0
Turnip	Canadian Gem	10	0	7
	Derby Bronze Green Top	10	0	7
Millet	Common	1	3	2
	Siberian	5	3	3
	Hungarian	0	—	—
	Japanese	0	—	—
Wheat	Marquis	1	0	0
	Red Fife	1	—	—
	Sun	1	0	—
Barley	Bark's	2	0	4
	Star	2	—	—
	Gold	2	—	—
Oats	Victory	2	0	0
	Banner	3	—	—
Corn	Longfellow	7	4	2
	Northwest Dent	4	4	2
Buckwheat	Japanese	6	2	2
Sunflower	Russian Giant	10	1	2
Rye Grass	English Perennial	2	1	1
Timothy		5	4	4
Carrot	Oxheart	9	0	7

*The minus sign indicates that no records were secured.

satisfactorily sterilized by a two- or three-minute immersion period in the disinfectant but periods of an hour or more were required in the case of seeds with hulls. In the case of oats and barley the germination was often

destroyed before aseptic growth was obtained. In order to allow time for the seeds to germinate before coming in contact with the fungus they were placed about half an inch from the advancing boundary of the mycelia. Check cultures consisted of duplicate lots of seed planted on sterile agar plates. The cultures were allowed to develop at 25° C., but owing to investigations indicating that *Rhizoctonia Solani* is most virulent at from 16° to 23° C. (*2*, *5*) a series was kept at room temperature which varied from 12° to 22° C. However, the degree of infection obtained in this series of cultures was comparable with that obtained in the cultures kept at 25° C.

In the second type of experiment the seedlings were grown in cold frames in pots inoculated by stirring into the soil cooked barley on which the *Rhizoctonia* had been grown. Before this inoculation, the soil was autoclaved for three hours at 20 lbs. steam pressure. Checks of uninoculated autoclaved soil were maintained.

In each experiment a minimum of 50 plants of each variety were carefully examined for lesions. Each seedling was given a pathogenicity value of from 0 to 10, determined by the lesions on the roots. The 0 figure represents complete freedom from lesions and the figure 10 complete destruction or coverage by lesions, 5 an intermediate condition, and so on. In the case of the seedlings in the pots an additional pathogenicity value of from 0 to 10 was given determined by the growth of the plant. The figure 0 represents normal growth and the figure 10 absence of growth. The results are given in Table 1.

FIGURE 1—The effect of *Rhizoctonia solani* Kuhn on the growth of clover and alfalfa. Top row—Red Clover (Chateaugay), Red Clover (Early Swedish). Bottom row—Alfalfa (Grimm), Mammoth White Dutch Clover.

In each case the pot on the left is a check, that on the right is inoculated with *Rhizoctonia*. Note the slight stunting of Early Swedish Red Clover and alfalfa.

It will be noted from the table that the figures obtained in the two experiments are not in accord: *e.g.*, clover seedlings were severely injured by the fungus when grown on agar but no noticeable inhibition of growth occurred when the same seed was planted in infested soil as can be seen in Figure 1. No explanation can be offered beyond suggesting that the resistance of clover to *Rhizoctonia* increases with age. Unpublished data[4] show that the water extracts of mature red clover roots inhibit the growth of *R. Solani* in pure culture.

When grown on the nutrient agar, wheat, barley, oats, rye grass and millet, with the exception of the Siberian variety, were quite resistant, while all the legumes, Swede turnips and sunflower were quite susceptible to attack by the fungus. Buckwheat, corn and timothy were somewhat intermediate.

Wheat, oats and rye grass, were likewise resistant to *Rhizoctonia* attach when grown on infested soil. Sunflower on the contrary was very susceptible when grown on the agar but was highly resistant when grown on infested soil. Timothy and corn seedlings were injured but not severely when grown on both infested agar and soil cultures.

The most noticeable feature of the pot experiment was the marked stunting of turnips and carrots although they were not visibly attacked by the fungus (Figure 2). The growth of the timothy in the inoculated

FIGURE 2—The effect of *Rhizoctonia solani* Kühn on the growth of swede turnips (Canadian Gem) Top row. Bottom row—Timothy and carrot (Oxheart).

[4] Ann. Report, 1932, Laboratory of Plant Pathology, Saanichton, B.C.

pots was also greatly reduced but in this case there were conspicuous lesions visible upon the roots. Barley was also stunted in the inoculated pots. In this case it was observed that sclerotia were so abundant that the roots were quite rigid for the first half inch or more from the seed although no definite lesions could be found. This condition also applied in the case of wheat and oats though the plants were not stunted. A few sclerotia were observed on the roots of all plants but were not sufficiently numerous to deserve comment.

With the possible exception of the Blue Bell pea, no definite evidence of varietal difference in susceptibility was observed. This variety was significantly less susceptible than Champlain or Capital when grown on the nutrient agar, and appeared to be slightly more resistant when grown in infested soil. Early Swedish Red clover was more resistant than Chateaugay or Mammoth White Dutch in the agar cultures. Although all the clovers were highly resistant as determined by the growth of seedlings on infested soil the Early Swedish variety was slightly stunted or apparently less resistant than the others.

It was thought that the marked stunting of carrots and turnips might be due to the more or less exclusive presence of the *Rhizoctonia* fungus in the soil for the inoculum was stirred into the soils shortly after they were taken from the autoclave. Accordingly, 36 eight-inch pots were filled with soil, 18 of which were sterilized in the autoclave for three hours at 20 lbs. pressure. Nine autoclaved pots and nine unheated pots were then uniformly inoculated by stirring in *Rhizoctonia* infested cooked barley seed; the others were kept as checks. The series therefore consisted of autoclaved soil inoculated with *Rhizoctonia*, unheated soil, and unheated soil inoculated with *Rhizoctonia*. The pots were sown with turnips (Canadian Gem) Carrots (Oxheart) and wheat (Marquis). Wheat was included for comparative purposes for in the previous experiment wheat was not significantly affected by the presence of *Rhizoctonia* in the soil.

TABLE 2.—SHOWING THE HEIGHT AT THE END OF SIX WEEKS OF WHEAT, TURNIPS, AND CARROTS, GROWING IN AUTOCLAVED SOIL, AUTOCLAVED SOIL INOCULATED WITH *Rhizoctonia*, UNHEATED SOIL AND UNHEATED SOIL INOCULATED WITH *Rhizoctonia*.

	Height in cms.		
	Wheat	Turnips	Carrots
Autoclaved soil	16	10	3.5
Autoclaved soil inoculated	10	4	1.5
Unheated soil	16	9	3.0
Unheated soil inoculated	10	4	1.5

The growth of these plants at the end of six weeks is shown in Table 2.

It is apparent from the data in Table 2 that the stunting of wheat, turnips and carrots was not due to the soil being more or less exclusively occupied by the *Rhizoctonia* fungus, for when unheated soil was inoculated with *Rhizoctonia* the growth of all these crops was inhibited. As in the previous experiment no lesions were found on the roots of turnips or carrots. An examination of the wheat seedlings from infested soil showed that many roots had turned brown at the tips and were dying back. This condition was similar to a stunting disease of cereals described by Samuel (7) which was caused by *Rhizoctonia*. No evidence of parasitism of wheat was

obtained in the first series reported in Table 1. In the series reported in Table 2, the evidence of parasitism of wheat in addition to turnips and carrots may be attributed to the greater humidity of the chamber of the greenhouse in which the seedlings were grown. The effect of humidity on the parasitism of *Rhizoctonia* is described by Park and Bertus (*4*) who found that this fungus is capable of infecting and killing young rice seedlings under humid conditions, but its activity is in a large measure inhibited under conditions of medium and low humidity.

SUMMARY

1. As judged by the growth of seedlings on infested soil, wheat, oats, red clover, crimson clover, and Mammoth White Dutch clover, appear to be immune or highly resistant. Alfalfa, sunflower, and rye grass are quite resistant, while peas, beans, vetch, buckwheat and timothy are moderately or highly susceptible to *Rhizoctonia Solani* Kühn.

2. Turnips and carrots are markedly stunted when grown on infested soil but exhibit no lesions or other symptoms of infection.

3. The growing of seedlings on a nutrient agar inoculated with *Rhizoctonia* is not a satisfactory method of determining their relative resistance to the fungus, *e.g.*, clovers and sunflower show maximum susceptibility when grown on agar but are highly resistant when grown on infested soil.

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Résumé

La Physiologie de *Rhizoctonia Solani* Kühn, III. Sensibilité de différentes plantes déterminée par l'infection des plantules. W. Newton et N. Mayers, Laboratoire fédéral de pathologie végétale, Saanichton, C.-B.

A en juger par la pousse des plantules sur terre infectée, le blé, l'avoine, le trèfle rouge, le trèfle incarnat et le grand trèfle blanc de Hollande, paraissent être réfractaires ou hautement résistants. La luzerne, les tournesols, et le ray-grass sont très résistants, tandis que les pois, les fèves, les vesces, le sarrasin et le mil sont modérément ou hautement sensibles au *Rhizoctonia Solani* Kühn. Les navets et les carottes cultivés sur sol infecté deviennent très rabougris, mais n'exhibent aucune lésion ni d'autres symptômes d'infection. La culture de plantules sur de l'agar nutritif, inoculé avec la Rhizoctonie, n'est pas un moyen satisfaisant de déterminer leur résistance relative au champignon; les trèfles et les tournesols cultivés sur de l'agar font preuve d'une sensibilité maximum mais ils sont hautement résistants lorsqu'ils sont cultivés sur sol infecté.

THE PHYSIOLOGY OF *RHIZOCTONIA SOLANI* KÜHN[1]

IV. THE EFFECT OF A TOXIC SUBSTANCE PRODUCED BY *RHIZOCTONIA SOLANI* KÜHN WHEN GRCWN IN LIQUID CULTURE, ON THE GROWTH OF WHEAT, CARROTS, AND TURNIPS

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The products excreted by fungi into the liquid media in which they are grown have been shown by many workers to exert conspicuous toxic effects upon living plant tissue. Liesau (*2*) found that *Didymella Lycopersici*, Sacc., the causal organism of tomato wilt, produced a toxin when grown in Richard's solution which caused a rapid wilting of cut tomato stems. Haymaker (*1*) observed that *Fusarium Lycopersici* Sacc. also produced a substance which caused a wilting of cut tomato stems. He also found a definite correlation between the pathogenicity of the latter organism and the toxicity of its by-products. That is, varieties of tomato which were resistant to attack by the fungus were likewise little injured by the excretory products. Other workers (*4*) have demonstrated the presence of toxins in the filtrate from liquid cultures of *Fusarium vasinfectum* Atk. and *Ophiobolus graminis* Sacc. which cause wilting and which inhibit the germination of seeds.

In a previous paper (*3*) we reported that turnips and carrots grown in pots containing soil infected with *Rhizoctonia Solani* Kühn were markedly stunted while wheat was not affected. As the stunted plants bore no visible signs of infection it was thought that the stunting might be due to some substance produced by the fungus which is toxic to turnips and carrots but not to wheat. Accordingly experiments were undertaken to determine the effect of the filtrate from liquid cultures of *Rhizoctonia* on the growth of these plants.

EXPERIMENTAL

A strain of *Rhizoctonia Solani* Kühn isolated by germinating the sclerotia on a Netted Gem potato was grown on Richard's solution[3] in large Erlenmeyer flasks for about three months, at room temperature (approx. 20° C.). At the end of this period the mycelial mats were removed and the medium filtered. The presence of a toxin in this filtrate was shown by the fact that turnip seedlings rapidly wilted when the normal or heated filtrates were added to the nutrient solution in which they were growing. When this toxin was found to be stable to heat, the filtrates from all the flasks were mixed, sterilized in the autoclave and maintained as a stock solution of toxin.

A preliminary experiment on the comparative resistance of wheat and turnip seedlings was performed by adding the filtrate to liquid cultures in which the seedlings were being grown. It was observed that the turnip seedlings wilted more rapidly and in the presence of more dilute concentrations than in the case of wheat.

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This greater resistance of wheat to the toxic factor compared with turnips was substantiated by the following experiment. Three-inch pots were filled with well washed sand, which had been previously sterilized in the autoclave for one hour at 20 lbs. pressure. The holes in the bottom of the pots were first sealed with paraffin in order to prevent any liquid from running through. They were then sown with wheat (Marquis), turnips (Canadian Gem) and carrots (Oxheart). The sand in each pot was saturated with 150 cc. of a nutrient[4] solution to promote growth.

When the seedlings were well established they were thinned out so that only ten remained in each pot. The filtrate was then added at the rate of 10 cc. each day for a period of 15 days. Checks were maintained by adding the same amount of the original sterile Richard's solution. The seedlings were allowed to grow for another seven days. They were then removed from the pots and the roots washed free from sand. The average dry weight of the roots and tops of the seedlings in each pot is shown in Table 1.

[3] *Richard's solution*

NH_4NO_3	10 gms.
KH_2PO_4	5 gms.
$MgSO_4$	2.5 gms.
$FeCl_3$	trace
Cane sugar	50 gms.
Water	1000 ccs.

[4] *Nutrient solution*

KNO_3	1 gm.
KH_2PO_4	1 gm.
$MgSO_4$	1 gm.
$Ca(NO_3)_2$	4 gm.
$FeCl_3$	trace
Water	1000 cc.

TABLE 1.—SHOWING THE INFLUENCE OF THE STERILIZED FILTRATE FROM A THREE MONTHS OLD CULTURE OF *R. solani* ON THE GROWTH OF WHEAT, TURNIPS AND CARROTS

	Wheat Wt. in gms.			Turnips Wt. in gms.			Carrots Wt. in gms.		
	Tops	Roots	Total	Tops	Roots	Total	Tops	Roots	Total
Check	.602	.420	1.022	.471	.163	.634	.141	.044	.185
Treated	.634	.477	1.111	.252	.055	.307	.099	.022	.121

It is seen from this table that the growth of carrots is reduced by about one-third and that of turnips about one-half by the filtrate. In contrast to this the growth of wheat treated with the filtrate is slightly but not significantly greater than the growth upon the check pots.

Owing to the difficulty of evaluating the effect of residual sugar and salts when filtrates from fungus cultures are added to plant cultures, it was thought desirable to prepare an extract in which the nutrients in the filtrate were reduced to a minimum. An extract of the washed and dried mycelium from liquid cultures of *R. solani* was prepared as follows: two grams of mascerated, dry mycelia was mixed with 100 cc. of water and cooked in the autoclave for 15 minutes at 20 lbs. pressure and then filtered.

Three dilutions of this extract, namely 1%, .5% and .25%, in the nutrient solution described above were prepared, as were also 50%, 25% and 12.5% solutions of the original filtrate. Wheat and turnip seedlings were started in nutrient solution and when well established were transferred to the prepared solutions. The experiment was performed in triplicate and ten seedlings were grown in each culture. After two weeks the seedlings were measured individually. The average heights are given in Table 2.

TABLE 2.—SHOWING THE EFFECT OF DIFFERENT CONCENTRATION OF THE FILTRATE FROM *Rhizoctonia* CULTURES AND OF THE AQUEOUS EXTRACT FROM THE GROUND MYCELIUM ON THE GROWTH OF WHEAT AND TURNIP SEEDLINGS

Concentration	Height of seedlings in cms.						
	Liquid culture filtrate			Mycelium extract			Check nutrient solution
	50%	25%	12.5%	1%	.5%	.25%	
Wheat	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Turnips	3.5	6.8	7.0	4.4	4.9	5.5	8.1

From the data it is apparent that the mycelial extract as well as the filtrate from old cultures contains a principle that is toxic towards turnips, but the growth measurements do not reveal any significant effect upon the wheat seedlings.

SUMMARY

Heat sterilized filtrates of old liquid cultures of *Rhizoctonia Solani* Kühn, were markedly toxic towards seedlings of carrots and turnips compared with wheat. Hot water extracts of washed, dried, and ground mycelia were likewise toxic towards turnips but at the concentrations employed in these experiments no evidence of toxicity towards wheat was obtained. This evidence suggests that a heat stable toxin is secreted by *R. Solani* during growth and that it is also present within the mycelia. This toxin may serve to evaluate the relative immunity of plant species and varieties against this fungus for previous studies showed that carrots and turnips were markedly stunted when planted on soils that were artificially infested with the living fungus. Under the same conditions the living fungus had little effect upon wheat.

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Résumé

La physiologie du *Rhizoctonia Solani* Kühn, IV. L'effet sur la végétation du blé, des carottes et des navets d'une substance toxique produite par *Rhizoctonia Solani* Kühn lorsqu'elle est cultivée en culture liquide. W. Newton et N. Mayers, Laboratoire fédéral de pathologie végétale, Saanichton, C.-B.

Des filtrés stérilisés par la chaleur de vieilles cultures liquides de *Rhizoctonia Solani* Kühn, se sont montrés très toxiques sur des plantules de carottes et de navets par comparaison au blé. De même, des extraits à l'eau chaude de mycélia lavés, séchés, et broyés, se sont montrés toxiques envers les navets, tandis qu'aux concentrations employées dans ces expériences aucune preuve de toxicité sur le blé n'a été notée. Cette constatation porte à croire que le *R. Solani* sécrète pendant sa croissance une toxine stable à la chaleur et que cette toxine est également présente dans le mycélium. Elle peut servir à évaluer l'immunité relative des espèces de plantes et des variétés contre ce champignon, car il a été démontré par des recherches précédentes que les carottes et les navets plantés dans des sols qui étaient infestés artificiellement du champignon vivant étaient très rabougris. Dans les mêmes conditions le champignon vivant n'avait que peu d'effet sur le blé.

SOFT ROT OF POTATOES CAUSED BY *PYTHIUM ULTIMUM* TROW[1]

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In October, 1930, a soft rot of potato tubers of the Netted Gem variety was observed on Salt Spring Island, British Columbia, during harvesting of the crop. On casual observation the majority of the diseased tubers appeared almost normal, with the skin intact. When pressed they were soft and resilient and on squeezing a watery liquid oozed out. The internal tissue was broken down and was dirty white to dark in colour, and gave off a fishy smell after exposure to the air. The majority of the affected tubers were not bruised or injured and infection appeared to have taken place initially at the stem ends. A similar condition has since been found on Vancouver Island, and in the Lower Mainland and Interior sections of the Province, indicating that it is widespread.

The disease has also been observed in potatoes stored at high temperatures and under poor ventilation. Under such conditions it spreads rapidly, often resulting in considerable losses. This usually occurs about planting time in spring when day temperatures begin to rise. The mean temperatures for Vancouver Island for fifteen years including 1932, during the months of April, May and June were 47°, 54° and 59° F. respectively.

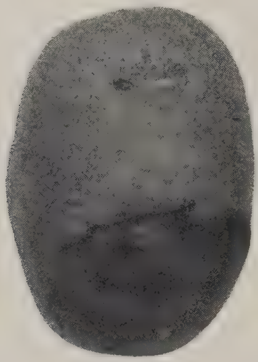

FIGURE 1—Soft rot of Netted Gem potato tuber caused by *Pythium ultimum* Trow.

FIGURE 2—Diseased Netted Gem tuber in section.

Another phase of the disease has recently been found to be associated with the rotting of cut sets in the soil immediately after planting in the spring. During recent years several growers on Vancouver Island and the Mainland have lost considerable acreages in this manner. The rotting of

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the seed has not been known to occur where whole sets were planted. This phase of the disease has been observed in potatoes planted on naturally drained light soils as well as on heavy textured soils. Samples of such soils were taken from the Interior of the province and from Vancouver Island and their pH values ranged from 7 to 8.2.

In some instances the affected sets break down completely appearing as shrivelled pieces in the soil. In others, only portions of the sets become affected. Germination is thus retarded and only weak plants are produced.

Other workers have reported a similar soft rot of potatoes. Thus Hawkins (*1*) has described the symptoms of "leak" disease in potato tubers caused by *Pythium de Baryanum* Hesse, which are similar to those described above for diseased tubers found during harvesting. Also Pethybridge and Smith (*2*) have described the symptoms of a watery wound rot causing serious damage to harvested potatoes. Their description of the disease was also similar to the above. They stated that the causal organism agreed in all essential features with *Pythium de Baryanum* Hesse, with the exception that no zoospores were observed.

Isolation and Pathogenicity

Several isolations have been made from infected tissue representing the different phases of the disease and in all cases *Pythium ultimum* Trow. was isolated.

The pathogenicity of the organism was established by inoculating freshly-cut tubers of the Netted Gem variety with inoculum from a young culture of the fungus and leaving them in a humidity chamber. Cut potato sets were also planted in sterilized soil mixed with a pure culture of the fungus. Positive infection resulted in both instances and the fungus was re-isolated. Whole tubers of the Netted Gem variety were also planted in inoculated soil but no infection was obtained.

When freshly cut sets were planted in inoculated soil in the greenhouse, symptoms of infection generally appeared within forty-eight hours as soft brown areas in the superficial cut tissue. In many of the sets, this infection was confined to the surface areas and germination took place normally. In other sets a considerable portion or all the tissue would be decayed, thus delaying or preventing germination.

Thirty sets of the Irish Cobbler variety were planted in the latter manner and after five days, it was found that fourteen were infected. Several of the diseased sets had developed sprouts and roots at the time of examination.

Five of the latter infected sets, in which the disease was well advanced, were re-planted in individual earthenware pots containing sandy loam soil. These were placed outdoors and after 70 days it was found that two of the sets were completely rotted. Plants had developed from the three other sets. Two of these plants were weak, being much smaller in size than normal. The old sets which had rotted were still attached to them. Similar conditions have often been observed in the field during the growing season. It was also noticed that there were numerous lesions caused by *Rhizoctonia solani* on the stems of these plants. The other plant was more normal in appearance and the attached old set was only partially decayed. On the stems of this plant no lesions caused by *Rhizoctonia solani* were present.

Varietal Susceptibility of Potato Tubers

Ten mature tubers of 15 different varieties were inoculated with the fungus from two day old cultures. Inoculation was done by cutting a section of tissue about 1 cm. deep from each tuber, inserting the inoculum and covering with moistened cotton wool, previously sterilized. The tubers were then kept in humidity chambers.

The varieties tested were Early Ohio, Early Epicure, Early St. George, Bliss Triumph, Irish Cobbler, Early Rose, Earliest of All, Beauty of Hebron, Wisconsin Pride, Gold Coin, Columbia Russet, Up-to-date, Manistee, Rural Russet and Rural New Yorker.

All the varieties proved to be susceptible. None of them exhibited any consistent difference in the degree of susceptibility.

Range of Hosts

Roots of mangels, beets and carrots were inoculated by the same method adopted in the last experiment. All of them proved to be susceptible to infection.

Effect of Temperature on the Growth of the Fungus

The effect of temperature on the growth of the fungus was determined by measuring the diameters of culture colonies grown on potato dextrose solid media at different constant temperatures. The approximate minimum temperature for growth is 4° C., the optimum between 25° and 31° C., and the maximum approximately 40° C.

Effect of Hydrogen Concentration of Culture Media on the Growth of the Fungus

The fungus was grown in petri dishes on potato dextrose agar solid media adjusted to different pH values. There was no growth at pH 2 and 3, and only slight growth when the media was adjusted to pH 3.8, indicating that the minimum pH for growth is between 3 and 4. There was slight growth on the media adjusted to pH 9, indicating that the maximum is above that figure. The optimum pH for growth is between pH 6 and 8.

Effect of Various Treatments of Cut Sets Against Infection

Potato tubers of the Irish Cobbler variety were taken and cut transversely. The different materials enumerated in Table 1 were then applied to the cut surfaces immediately after cutting, the solid materials being first crushed into powder form, and then dusted in even layers on the cut surfaces with a camel's hair brush. In the mercuric chloride paste series, the cut surfaces of the sets were immersed in the paste until even layers were deposited.

Uniform pieces of inoculum from young cultures of the fungus were then placed on the treated and untreated sets. These were kept in humidity chambers and observations on the degree of infection and injury to the tissue were made after four days. The results are given in Table 1.

The mercuric chloride and infusorial earth paste was prepared by adding 100 ccs. of the mercuric chloride solution to 40 grams of powdered infusorial earth and mixing well. These proportions were found to give satisfactory results for immersion. The mercuric chloride-infusorial earth dusts were obtained by drying the paste prepared in the above manner. The concentrations of the mercuric chloride solutions are given for each series in Table 1.

TABLE 1.—THE EFFECT OF TREATING CUT POTATO SETS AGAINST INFECTION BY *Pythium ultimum*

Material	No. of sets treated	No. sets infected after 4 days	Tissue injury due to treatment 0–10
Copper sulphate	10	0	10
Copper sulphate mixed with infusorial earth ratio 50 : 50	10	0	10
Bordeaux dust $CuSO_4$ 16 CaOH 100	10	5	2
Copper carbonate	10	0	2
Copper carbonate mixed with infusorial earth 50 : 50 ratio	10	2	1
Calcium sulphate	10	9	0
Sulphur	20	12	0
Infusorial earth	20	17	0
Mercuric chloride solution 1 : 500	26	0	0
Mercuric chloride solution 1 : 1000	24	6	0
Mercuric chloride infusorial earth dust 1 : 1000	10	3	0
Mercuric chloride infusorial earth dust 1 : 100	10	0	1
Cut sets dried	10	8	
Cut sets allowed to callus in humid atmosphere at room temperature for 48 hrs.	30	1	
Check—freshly cut sets	20	18	

Analysis of Table 1 shows that there was severe injury of the tubers when treated with copper sulphate, the sets being finally killed. There was also slight injury when Bordeaux dust and copper carbonate were applied. Owing to tissue injury these compounds were discarded for further experimentation.

Soil Infection Studies

In order to find a method of checking infection of tubers in the soil, further work was done. For this purpose sandy loam soil was sterilized, and well mixed with a pure culture of the fungus grown on cooked barley seed. Several infected tubers were also broken up and well mixed with the soil. This inoculated soil was then laid out on a bench in the greenhouse and kept moistened. Mature tubers of the Netted Gem variety were used throughout the experiment. The tubers in each series were cut longitudinally, making two sets for each tuber, each set varying from 2

to 5 oz. Some of the sets were then treated by dusting or by immersion in the pastes and liquids as recorded in Table 2. They were then planted immediately in the inoculated soil. The untreated checks were also planted immediately after cutting. In series 7, the cut sets were placed on moistened peat moss in a humidity chamber in the greenhouse where the temperature ranged between 50° to 85° F. After forty-eight hours they were taken out and planted immediately. An even unbroken callus had been formed by that time. In series 8, the cut sets were left exposed on a bench in the greenhouse for forty-eight hours after which time they were rather soft and shrivelled. They were planted when in that condition.

Table 2.—The effect of various treatments of planted cut potato sets against infection by *Pythium ultimum*

Material	Method of treatment	No. of sets	No. of infected sets	Remarks
1. Sulphur	Dusted	20	15	
2. Mercuric chloride solution 1 : 100	Immersion for 5 min.	20	0	Moderate surface injury.
3. Mercuric chloride solution 1 : 500	Immersion for 5 min.	20	6	Slight surface injury.
4. Mercuric chloride solution 1 : 1000	Immersion for 5 min.	20	9	No injury.
5. Mercuric chloride infusorial earth paste 1 : 100	Immersion for 30 sec.	20	6	Moderate surface injury.
6. Mercuric chloride infusorial earth paste 1 : 500	Immersion for 30 sec.	20	6	Slight injury.
7. Cut sets allowed to callus at room temperature in humid atmosphere for 48 hours		20	0	
8. Cut sets allowed to dry out for 48 hrs. at room temperature		20	18	Surface tissue badly contaminated with secondary organisms.
9. Whole tubers		20	0	
10. Check—freshly cut sets.		20	16	

In order to procure further data on the effectiveness of different treatments, potato tubers of the Netted Gem variety were cut transversely into slices of approximately 2 cms. in thickness. Both surfaces of each slice were afterwards treated in the manner described in Table 3. During the twenty-four hour period before planting in series 1, 2, and 3, the slices were left exposed at room temperature in the laboratory. All the slices were planted in infected soil and after four days, observations on the degree of infection were made. By slight infection in Table 3, is meant that only the surface cut tissue was affected, while by severe infection is meant that most of the tissue had broken down.

TABLE 3.—THE EFFECT OF VARIOUS TREATMENTS OF PLANTED POTATO SLICES AGAINST INFECTION BY *Pythium ultimum*

Series	No. of potato slices	Slight infection	Severe infection
1. Dusted with sulphur 24 hours before planting	100	23	1
2. Immersed in mercuric chloride-infusorial earth paste 1 : 500, 24 hrs. before planting	150	11	120
3. Immersed in mercuric chloride solution 1 : 500 for 5 min., 24 hrs. before planting	100	2	80
4. Potato slices allowed to form a callus in humidity chambers at room temperature, 48 hrs. before planting	150	4	5
5. Check—planting done immediately without treatment	70	2	68

Effect of Soil Moisture on Degree of Infection

In order to determine the effect of soil moisture on the incidence of infection, potato slices were cut as in the previous experiment. Some were planted immediately in moist and dry infected soil, while others were allowed to form a callus as in series 4, Table 3, before planting. Observations were made four days after planting and the results are given in Table 4.

TABLE 4.—THE EFFECT OF SOIL MOISTURE ON THE DEGREE OF INFECTION OF POTATO SLICES BY *Pythium ultimum*

		Percentage moisture	No. of potato slices	No. of infected slices
Freshly cut potato slices	Moist	22	50	50
	Dry	8	50	44
Potato slices allowed to form a callus in a humid atmosphere at room temperature for 48 hours	Moist	22	46	5
	Dry	7	45	1

It is evident from the above results that infection of freshly cut sets can take place in very dry soils.

CONCLUSIONS

It is evident from the data presented that the storage temperature for potatoes should be approximately 4° C. or below, since the minimum temperature for the growth of the fungus is approximately 4° C. This is important during the spring months when the disease is known to be present.

Further analysis of the data shows that the various treatments of cut sets with mercuric chloride are of little value to prevent infection. When the cut sets were dusted with sulphur and planted immediately, there was considerable infection, but when these dusted sets were allowed to dry out at room temperature for twenty-four hours before planting the infection was reduced. In the latter case the sulphur apparently assists tissue suberization, for the sulphur itself is not toxic to the fungus. The cut sets that were allowed to dry out before planting were severely infected and badly contaminated with other secondary organisms. Such sets did not develop any sprouts or roots after planting. Freshly cut sets that were planted in soil containing the fungus, almost invariably became infected. In all the experiments where whole uninjured tubers were planted there was no infection. When cut sets were allowed to form a callus in a humid atmosphere at room temperature for forty-eight hours before planting, infection was reduced greatly. It is evident that of the methods investigated this is the best for treating cut sets before planting to avoid infection. The various chemical treatments are apparently of little use except inasmuch as they may assist in tissue suberization. Priestly and Woffenden (*3*), reporting on the healing of wounds in potato tubers stated that cut sets should be left in a warm, damp and shady atmosphere so as to form a continuous suberized layer, this layer being formed within twenty-four to forty-eight hours. A few days later, cork is formed below this suberin deposit as a result of cell divisions in the cork phellogen layer. They further stated that in a dry atmosphere, especially in sunlight, the suberin layer formed may not be continuous.

It is obvious from this that the principle of proper suberization is important in dealing with cut potato sets that are to be used for planting. This was done quite effectively in the laboratory by laying cut sets on moist peat moss and covering with clean wet bags. These were kept moist by sprinkling with water occasionally. Again, according to Priestly and Woffenden (*3*) "the deposit of fatty suberin layer is formed by oxidation and condensation of fatty substances depositing from the sap drying up in the tissues at the cut surface." It is evident from this that a necessary condition for proper suberization is access to a good supply of oxygen. The cut tubers therefore should have a sufficient supply of air when allowed to callus over in the manner described.

Infection was found when freshly cut sets were planted in moist as well as in very dry soils, but there was a slightly higher percentage of infected sets found in the moist soil. This experiment indicates that when the fungus makes contact with freshly cut tissue, infection may result, regardless of the moisture content of the soil. Fungus growth, however, may be stimulated in soil which is too moist, increasing the chances of infection. In the interior sections of the Province where surface irrigation is practised the soil moisture during planting should be ample for immediate growth of the tubers so as to avoid irrigation immediately after planting.

SUMMARY

1. A soft rot of potato tubers caused by *Pythium ultimum* Trow. is described. It has been found in tubers during harvesting, in storage and also in cut sets after planting in spring.

2. The disease is fairly widespread in British Columbia and is causing considerable losses in some districts, particularly after planting in spring.

3. The pathogenicity of the fungus has been established by direct inoculation on freshly cut tubers and by planting freshly cut tubers in inoculated soil. Infection is very rapid at the optimum temperature for the growth of the fungus. Complete disintegration of the internal tissue may take place within four days under the latter condition.

4. The optimum temperature for the growth of the fungus is between 25° and 31° C. The minimum and maximum temperatures for growth are approximately 4° and 40° C. respectively.

5. The optimum hydrogen ion concentration for the growth of the fungus is between pH 6 and 8. The maximum is above pH 9 and the minimum between pH 3 and 4.

6. The disease susceptibility of fifteen different potato varieties was determined. All of them proved susceptible to infection.

7. The fungus was found to cause disintegration of the roots of mangels, beets and carrots.

8. Whole uninjured tubers did not become infected when planted in soil inoculated with the fungus.

9. Treating cut sets with mercuric chloride solutions and pastes of different concentrations did not effectively check infection.

10. Dusting the cut sets with sulphur and planting immediately in infected soil did not check infection, but when the dusted sets were not planted for twenty-four hours after treatment, the infection was reduced.

11. Cut sets that were allowed to dry out for forty-eight hours before planting, were readily attacked by the fungus as well as by secondary organisms.

12. When cut sets were allowed to form a callus at room temperature in an atmosphere of high humidity for forty-eight hours before planting infection was reduced greatly.

13. Infection of freshly cut sets can take place in wet and very dry soils. There was slightly less infection in a very dry than in a moist soil.

ACKNOWLEDGMENT

The author wishes to thank Dr. Charles Drechsler, Bureau of Plant Industry, Washington, D.C., for identifying the causal organism, and also Mr. H. S. MacLeod, District Plant Disease Inspector, for his co-operation in the work.

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Résumé

Pourriture molle des pommes de terre causée par le *Pythium ultimum* Trow. Walter Jones, Laboratoire fédéral de pathologie végétale, Saanichton, C.-B.

L'auteur décrit une pourriture molle des tubercules de pommes de terre causée par *Pythium ultimum* Trow. Cette pourriture a été trouvée également dans des tubercules au cours de l'arrachage, en cave ainsi que dans les plantons, ou fragments de tubercules, après la plantation, au printemps. Elle est assez répandue en Colombie-Britannique où elle cause des pertes considérables dans certains districts, spécialement après la plantation au printemps. On a établi la pathogénicité du champignon par une inoculation directe sur les tubercules fraîchement coupés et en plantant les tubercules fraîchement coupés dans la terre inoculée. L'infection est très rapide à la température optimum pour la végétation du champignon. Dans ces conditions, une désagrégation complète des tissus de l'intérieur peut se produire en quatre jours. La température optimum pour la végétation du champignon est entre 25° et 31° C. Les températures minimum et maximum pour la végétation sont environ 4° et 40° C., respectivement. La concentration optimum d'hydrogène-ion pour la végétation du champignon est entre pH 6 et 8. Le maximum est supérieur à pH 9 et le minimum est entre pH 3 et 4. La sensibilité à la maladie de quinze variétés différentes de pommes de terre a été déterminée. Toutes ces variétés se sont révélées sensibles à l'infection. Il a été constaté que le champignon cause une désagrégation des racines des betteraves fourragères, des betteraves potagères et des carottes. Les tubercules entiers non endommagés plantés dans un sol inoculé avec le champignon ne se sont pas infectés. Le traitement des plantons avec des solutions de chlorure de mercure et des pâtes de différentes concentrations n'a pas enrayé l'infection d'une façon efficace. Le saupoudrage des plantons avec du soufre et leur plantation immédiate dans une terre infectée n'a pas enrayé l'infection, mais lorsque les plantons saupoudrés n'étaient plantés que vingt-quatre heures après le traitement, l'infection était réduite. Les plantons qu'on laissait sécher quarante-huit heures avant la plantation étaient promptement attaqués par le champignon ainsi que par les organismes secondaires. Lorsqu'on laissait les plantons former un callus à la température de la chambre dans une atmosphère très humide pendant quarante-huit heures avant la plantation, l'infection, était grandement réduite. Les fragments fraîchement coupés peuvent s'infecter dans des sols humides et très secs. Il y avait un peu moins d'infection dans un sol très sec que dans un sol humide.

INVESTIGATIONS ON THE BLACK KNOT OF PLUMS AND CHERRIES

III. SYMPTOMATOLOGY, LIFE HISTORY, AND CULTURAL STUDIES OF *DIBOTRYON MORBOSUM* (SCH.) T. and S.[1]

L. W. KOCH[2]

INTRODUCTION

Black knot is undoubtedly the most destructive fungous disease attacking plums in North America and though common on sour cherries is usually less severe. In Ontario the injury caused by this fungus varies considerably from year to year both as to host and locality. In the Niagara peninsula where spraying and pruning are commonly practised and where relatively few wild hosts are present black knot is not as serious a factor in the growing of plums and cherries as it is in the Georgian Bay and certain other districts.

Certain phases of the present investigations on black knot have been reported in previous articles (*9*), (*10*), and while the articles III and IV submit the results of the remaining fundamental phases of the problem they also include a general summary of all findings, thereby completing the report of the results of the investigations to date.

REVIEW OF LITERATURE

Only literature relevant to those phases of the problem considered in the present article will be dealt with here. The early investigators of this disease, *e.g.*, Peck (*12*), Farlow (*5*), Halsted (*6*), Beach (*1*), etc., contributed largely to our present day knowledge of the symptomatology of black knot. Since that time numerous brief popular articles have appeared all of which deal chiefly with symptomatology and suggestions for control, but none of these has added to our knowledge of symptomatology. In a recent article (*9*) dealing with the present investigations it was demonstrated that the pink areas which appear consistently in July and August every year on the conidial stroma of the knot are caused by *Cephalothecium roseum* Corda. It was proved that the latter organism actively parasitizes the stroma of the black knot pathogen and by destroying the perithecial initials exerts an important measure of biologic control over the knot pathogen. Certain other fungi were also found to be constantly associated with knots and to alter the normal development of perithecia on the knots during the winter. Concerning the life history of the causal organism and other fundamental phases of the problem, contributions have remained few in number. Heald (*7*) in 1934 states that "with our present information there are several blanks in the life history of the pathogen, the part played by the different spore forms and the exact time and manner of infection being based on observational data." During the course of the present investigations (*9*) it has been reported that "over a period of four years the initial ascospore discharge from perithecia of *D. morbosum* on *Prunus domestica* L. in the Niagara peninsula varied from March 23 to

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April 6, after which discharges occurred periodically until June 6 or 7." Under laboratory conditions perithecia from the same host discharged ascospores as early as November 20 in 1931.

Regarding the life history of the pathogen reports have been both confusing and contradictory. No investigator has successfully determined the life history of *D. morbosum* by the germination of monoascospores in pure culture or by infection experiments on the host. Farlow (*5*) in 1876 described three types of pycnidia which he found mingled with perithecia. At least one of these, namely *Hendersonula morbosa* Sacc., has been proved (*10*) to be not genetically connected with *D. morbosum*. Later Humphrey (*8*) in 1891 stated that he obtained a single-celled, brown-spored pycnidial form from ascospores. Undoubtedly this fungus was the *Coniothyrium* sp. which was shown in a previous article (*10*) to be an organism distinct from the black knot pathogen though closely associated with it throughout all but the most incipient stages of its life history. The *Coniothyrium* sp. is also saprophytic on buds and bark of various fruit trees. Stevens (*16*) in 1925 assigned the conidial stage of the black knot pathogen to the genus *Cladosporium*.

There is no literature dealing with the study of *D. morbosum* in artificial culture.

SYMPTOMATOLOGY

Though the outstanding symptoms of black knot are familiar to the majority of phytopathologists, certain of them must be discussed here because of the inadequate and in part incorrect symptomatological picture prevailing in text-books.

Black knot is first manifested as a small swelling on a twig or branch either at the border of a knot already formed or separate from it. In the case of primary infections, swellings nearly always appear during the fall of the year of infection. During the following spring all knots become much larger in size, the bark ruptures and during June the surface of the gall-like excrescence becomes covered with a velvety-green pad of fungous tissue. During midsummer pink and white areas caused by *Cephalothecium roseum* and other fungi (see Review of Literature) frequently appear on the green conidial stroma of many knots. During late summer and early fall the green pad of mycelium disappears and is replaced by black stromatal tissue from beneath. By late fall most knots are perfectly black. During the winter perithecia of *D. morbosum* mature on the surface of the knots and ascospores are discharged during April and May.

The opinion regarding the first appearance and subsequent development of knots has been that in most cases knots do not become conspicuous until the spring following infection. Even though there are records of the appearance of small swellings in the fall of the year of infection no conidia have been observed until the following year. These observations have given rise to the opinion universally expressed in text books, namely, that two years is required for *D. morbosum* to complete its life cycle on the host. However, the present investigations have proved that this is not always true. Observations made during the fall and spring of three consecutive years proved that at least 93% of all knots are visible as swellings during the fall of the season of infection, while the remaining 7% were doubtful.

PLATE I.—1. Branch of *P. domestica* one and one-half years old showing numerous tiny knots on current season twigs. Photo taken 12.9.32. 2. Current season twigs with knots already producing conidia. Photo taken 25.8.32. 3. Mature black knot producing perithecia of *D. morbosum*. Photo taken 24.4.33. 4. *Hormodendrum* sp. (strain C) growing on sour cherry fruits. 5. As in 4, strain X. 6. Photomicrograph of germinating ascospore of *D. morbosum*. This ascospore developed into conidial colony, strain Z, × 300.

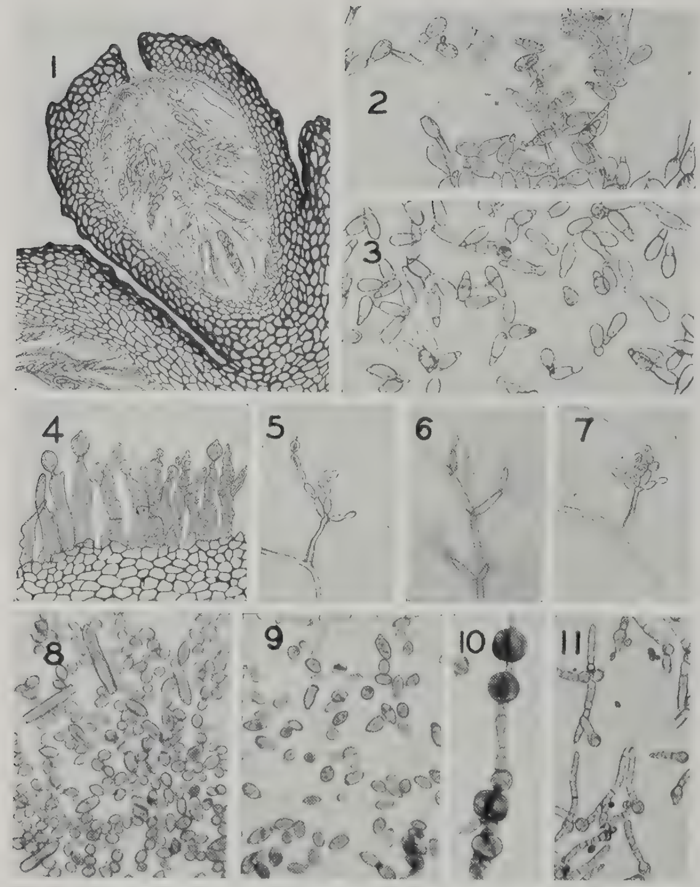

Plate II, Life history of *D. morbosum*.—1. and 4. Drawings; 2, 3 and 5-11, Photomicrographs. 1. Mature perithecium of *D. morbosum*, × 140. 2. Ascospores of *D. morbosum* from knot on *P. pennsylvanica*, × 300. 3. As in 2, from knot on *P. domestica*, × 300. Note difference in shape of spores in 2 and 3. 4. Conidial stage on host, × 500. 5-7. Various types of conidiophores and conidia (*Hormodendrum* sp.) in culture, × 300. 8. Conidia of *Hormodendrum* sp. (strain Z) from 7-day old culture, × 300. 9. As in 8 (strain C). 10. Chlamydospores of *D. morbosum*, × 300. 11. Conidia of *Hormodendrum* sp. (strain X) germinating, × 300.

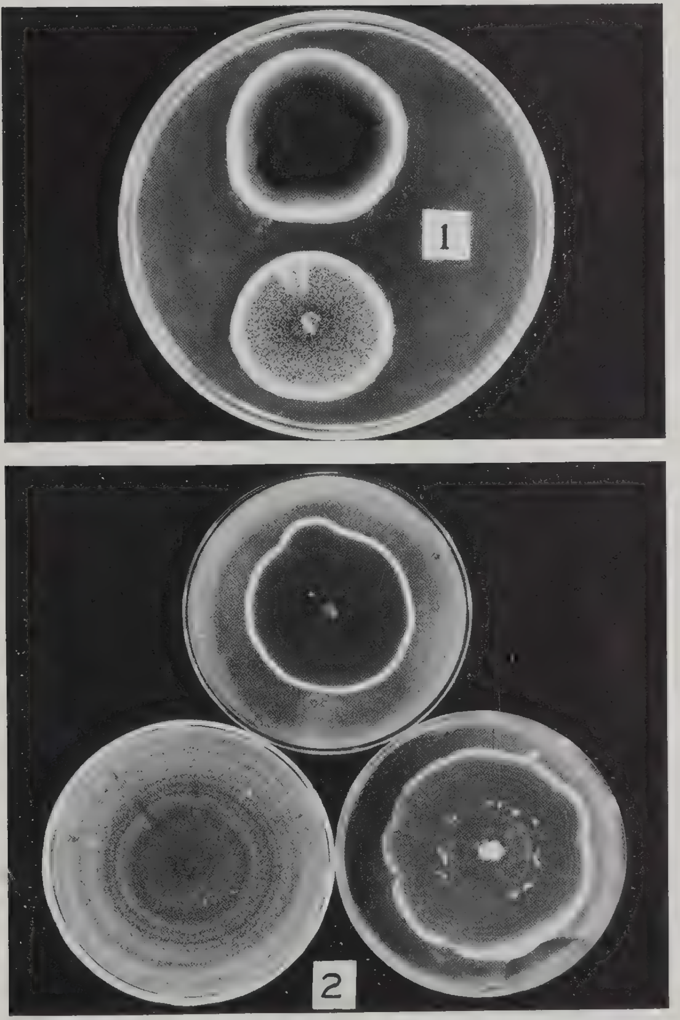

PLATE III.—Petri dish colonies of conidial (*Hormodendrum*) strains of *D. morbosum* growing on potato $2\frac{1}{2}$ per cent dextrose agar. 1. (colonies 33 days old at 5-7° C.) (Upper, strain Z; Lower, strain X. 2. (Colonies 19 days old at 22-24° C.) Upper, strain Y; Lower left, strain Z; Lower right, strain X. Three strains in 2 all isolated from monoascospores obtained from a single knot.

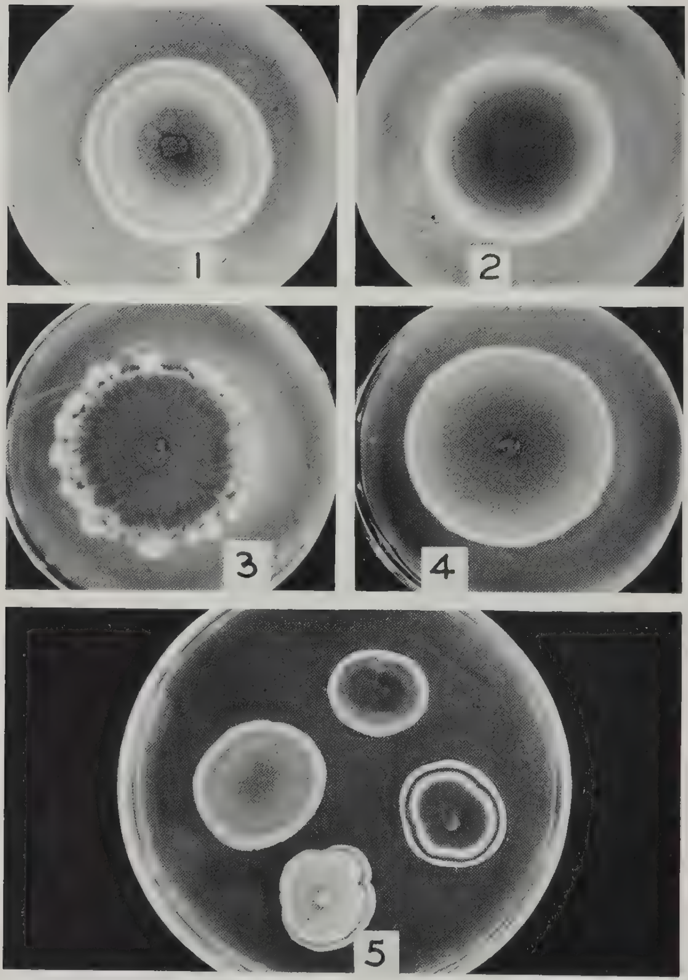

PLATE IV.—Four conidial (*Hormodendrum*) strains isolated from monoascospores of *D. morbosum*. 1-4. Colonies 14 days old growing on potato 2½ per cent dextrose agar at 27-28° C. 1. Strain C. 2. Strain Y. 3. Strain X. 4. Strain Z. 5. Colonies 33 days old growing on potato agar at 8-10° C. Upper, strain Z; Middle left, strain X; Middle right, strain Y; Lower, strain C.

Of these knots which were visible during the fall, a proportion, which varied greatly from year to year, always produced conidia during August and September of the year of infection (Plate I, 2). Usually the area producing conidia was small but even so is capable of producing a large number of spores. On the conidial-bearing stromata of these knots perithecia developed in many instances during the subsequent winter and spring, thereby completing the life cycle of the fungus within one year.

The general opinion that wood of any age is susceptible to primary infection has not been substantiated by the present investigations. A survey conducted during three consecutive years revealed that in a plum orchard containing numerous varieties 95% of all newly-formed knots originated on current-season twigs, 1% originated on branches more than one year old, 1% on relatively fresh pruning stubs and the remaining 3% were of doubtful origin. The remarkable feature of the survey was, however, the preponderance of knots originating on current-season twigs (Plate I, 1). During the course of the survey numerous knots were observed on the large branches and in some cases on the trunks themselves, but continuous observations of numerous tagged knots proved that in practically all cases the invasion of the trunk could be traced to infection through a small lateral.

Smaller twigs when infected were frequently killed within a year after infection but because of the localized effect of the parasite, larger branches usually resisted the attack of the fungus for several years before succumbing. In June and July, during which months most of the hypertrophy of the host occurred many of the smaller twigs and branches wilted suddenly, due to the complete interruption of the water supply by the knot.

TAXONOMY

The pathogen which causes black knot was first named *Sphaeria morbosa* by Schweinitz (*15*) in 1821. Other generic names have been suggested since that time but without general acceptance. Saccardo (*14*) assigned the organism to the Dothideales and called it *Plowrightia morbosa* (Sch.) Sacc.; Plowright (*13*) thought it belonged in the genus *Gibbera;* Ellis (*3*) called it *Cucurbitaria morbosa* (Sch.) Ell., and soon afterwards Cooke (*2*) called it *Othiella morbosa* (Sch.) Sacc. Ellis and Everhart (*4*) in 1890 adopted Cooke's nomenclature and termed it *Othiella morbosa*, claiming that the genus *Plowrightia* in Sacc. Syll. is unsatisfactory, including as it does, species with true perithecia, such as *Sphaeria morbosa* (Sch.) and others with mere ascigerous cells, such as *Dothidea ribesia*, (Pers.) the latter being a genuine *Dothidea.*

Plowrightia morbosa appears to have been used more widely than any other name in referring to the pathogen. However, in recent years there has been some uncertainty as to the correct taxonomic position of this fungus. Thiessen and Sydow (*18*) in 1918 placed the fungus in their newly-proposed order the Pseudosphaeriales and named it *Dibotryon morbosum* (Sch.) T. and S. In 1921 Sorauer (*17*) transferred the genus *Plowrightia* Sacc. to *Botryosphaeria* and named the black knot fungus *Botryosphaeria morbosa* (Sch.) Sor.

The perithecia of the pathogen in question have individual perithecia with distinct walls (Plate II, 1) and do not at all resemble those of the

type species *Plowrightia ribesia* (Pers.) Sacc. where the perithecia are reduced to polyascous loculi. The fungus cannot, therefore, be placed in the genus *Plowrightia* Sacc., which, in any case is a synonym of *Dothidella* Speg. On the other hand, the fungus is not typically a sphaeriaceous one but rather Pseudosphaeriaceous in character. It would, therefore, be most advisable to call it *Dibotryon morbosum* (Sch.) T. and S. though it is still questionable whether or not Thiessen and Sydow (*18*) were justified in ranking the Pseudosphaeriales as a separate order.

LIFE HISTORY STUDIES

Investigations concerning the life history of the fungus causing black knot were conducted to obtain information concerning (1) the characters of the perfect stage and (2) the correct imperfect stage or stages. In the case of the latter, as explained previously, numerous and widely-different imperfect stages have been reported for *D. morbosum* none of which has been proved experimentally.

Material was collected from scattered localities throughout Ontario, and specimens were also obtained from Manitoba, Quebec and Nova Scotia. This material was utilized without special preparation and in most cases sections were cut with a sliding microtome after which they were stained in cotton blue and mounted in lacto-phenol for examination.

Perithecial Stage

D. morbosum was observed to be constantly associated with knots collected during the winter, though there are marked variations in the abundance of its perithecia on different knots even on the same tree. Mature ascospores can usually be found as early as February on *Prunus domestica* in Ontario though they apparently do not begin discharging ascospores in the Niagara peninsula until late in March or early in April (*9*). Examinations of the perithecia and ascospores showed that they corresponded in detail with Saccardo's (*14*) description of *Plowrightia morbosa*.

Isolations from the Perithecial Stage

Using material from the localities mentioned above, numerous isolations were made from the perithecial stage of *D. morbosum*. Pure suspensions of ascospores were obtained by the method described in a previous article (*9*). Loopfuls of these suspensions were streaked over the surface of Petri dishes containing potato 2.5% dextrose agar or other solid media. In this way all ascospores were in the same plane and could be examined when desired.

To obtain information regarding germination, the suspensions of ascospores were subjected to various conditions of temperature and moisture before plating. In all, 142 isolants were obtained from perithecia of *D. morbosum*, 48 of which were monosporous in origin. From all monoascosporous cultures a species of *Hormodendrum* developed. There were, however, four apparently distinct strains designated X, Y, Z and C (Plates III and IV) which will be dealt with in detail later.

It should be mentioned in connection with the above isolations that great difficulty was experienced in obtaining ascospores which would both germinate and complete their development to an imperfect stage. After

48 to 96 hours in distilled water the ascospores of *D. morbosum* frequently produced germ tubes of considerable length, in accordance with illustrations and statements by previous investigators (*5*, *7*), but in most cases their development ceased at that point. It was discovered that ascospores germinated only when discharged from fully mature perithecia. Frequently it was necessary to allow the spores to germinate and remain in distilled or tap water for 3 to 5 days before plating them in order that some of them might continue growth. In a few cases ascospores which were allowed to remain in a thin film of water for a week continued development and produced conidia after very little mycelial development. Low temperatures of 7-12° C. for the above-mentioned period prior to plating also frequently improved the percentage germination. Provided the ascospores were mature an increase of 50% in the oxygen of the atmosphere surrounding the thin film of water containing the ascospore suspension improved to some extent both the germination and further development of the ascospores.

Close examinations and measurements of ascospores revealed the existence of a relation between size of ascospores and host, worthy of mention. The lengths and widths of 388 ascospores (in a water suspension) from knots on each of the hosts *Prunus pennsylvanica* and *P. domestica* were taken and the percentages of the total number of ascospores of each size unit were calculated. The latter are shown graphically in Figure 1. It will be observed in the graph that the ascospores from knots on *P. pennsylvanica* are both longer and wider than those from knots on *P. domestica*. While the modes for the length and width of the ascospores on the former host lie at 17 to 18, and 7 respectively, for ascospores on the latter host the modes lie at 16 and 6 respectively. The difference in size

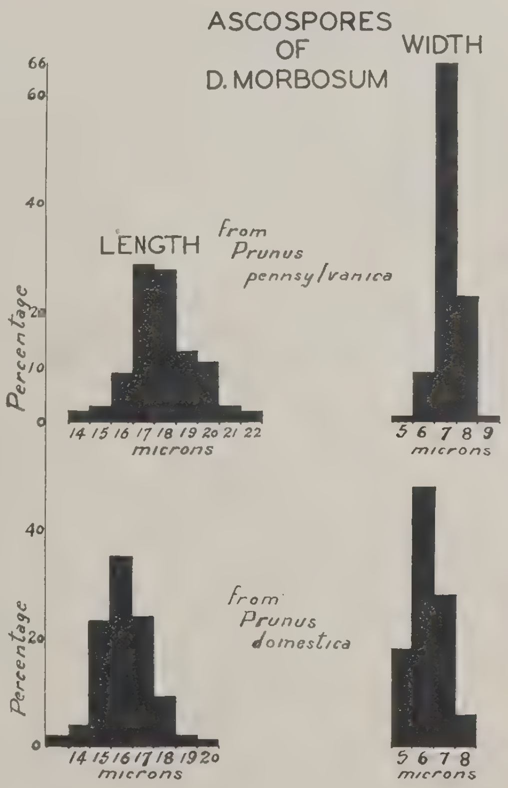

FIGURE 1—Graph illustrating distribution curves of sizes of ascospores of *D. morbosum* from *Prunus pennsylvanica* and from *P. domestica*.

and shape of ascospores from knots on these two hosts is illustrated in Plate II, 2 and 3.

During the investigations involving the germination of ascospores certain interesting cultures were obtained in April, 1933. On three occasions from monoascospores of *D. morbosum* and on eight other occasions from a suspension of supposedly-pure ascospores, conidial cultures were obtained which resembled the *Hormodendrum* stage of *D. morbosum* in every respect except that both mycelium and spores were hyaline, and the mycelium was somewhat less in diameter and less vigorous than that of the *Hormodendrum*. The colonies themselves were pure white on potato dextrose agar and invariably with age and repeated transfers they ceased to produce conidia. It would seem highly suggestive that these cultures were saltants from the parent, in which loss of colour resulted during reduction division in the formation of the ascospores.

Conidial Stage

As stated above in the Review of Literature numerous conidial stages have in the past been reported for *D. morbosum*. During the present investigation, however, in two instances germinating ascospores were photographed at intervals of two hours during the initial stages and up to eight hours during their later development until they finally developed conidia typical of *Hormodendrum* 68 to 74 hours later (Plate I, 6).

In addition to the latter proof that *Hormodendrum* is the imperfect stage of *D. morbosum*, reference to the pathogenicity studies in article IV will show that in several instances inoculations with either ascospores of *D. morbosum* or conidia of *Hormodendrum* sp. resulted in the development of knots, some of which produced a *Hormodendrum* conidial stage followed by perithecia belonging to *D. morbosum*.

Summing up the foregoing evidence we find definite proof of the genetic connection of *Hormodendrum* sp. and *D. morbosum*.

Mention should be made of the difference in morphology of the conidial stage of *D. morbosum* on its host and in culture. On its host conidia are produced superficially on green pads of stroma. Conidiophores are shorter and much more flexuose than in culture, being erect, septate, usually simple and ranging in size from 20–65 × 4–6 microns (Plate II, 4). Conidia are borne singly, usually at the apex of the conidiophore, obovate, unicellular, light olivaceous brown and 4–8 × 2–5 microns in size.

On the other hand, in culture conidiophores of the pathogen vary tremendously in length (Plate II, 5–7), frequently being more than 100 microns in length, and long dendroid chains of conidia develop at the apex. Conidia varied in size from 3–20 × 2–5.5 microns (Plate II, 8 and 9).

When portions of the conidial-bearing stroma of a knot were brought indoors and placed in moist Petri dishes a certain number of the conidiophores increased in length and developed chains of spores very similar to those produced on artificial media. No doubt, wind and lack of moisture play an important role in the morphology of the fungus outdoors on the host.

Isolations from the conidial stage

Monosporous cultures were made from *Hormodendrum* conidia, employing the same methods as were used for the ascospores. These were made both from *Hormodendrum* conidia in cultures which originated from ascospores and from conidia which developed on the fruiting surface of knots in nature during June. In every case colonies of *Hormodendrum* sp. developed which were similar in every respect to those originating from ascospores. The same four strains which developed from ascospores appeared frequently in these cultures. Frequently three of these four strains were isolated from a single knot on either *Prunus domestica*, *P. cerasus* or *P. pennsylvanica*. Two additional strains appeared on several occasions from conidia but since certain saprophytic species of both *Hormodendrum* and *Cladosporium* were also frequently isolated from the conidial fruiting surfaces of knots these strains were not intensively investigated.

Isolations from Swollen Tissues Adjacent to Knots

More than 600 isolations were made from swollen tissues adjacent to knots and from apparently normal tissues nearby. In these isolations a species of *Coniothyrium* frequently appeared, in addition to the *Hormodendrum* sp. This *Coniothyrium* sp. has been discussed in a previous article (*10*) and has been definitely proved not to belong genetically to *D. morbosum*. During the more recent investigations in which young knots were detected when they appeared as only very faint swellings on infected twigs (Plate I, 1) at which time infections could not have been more than 4 months old, it was found that only *Hormodendrum* sp. appeared in the isolations, provided epidermal tissues were removed prior to placing the tissues on media. It was frequently difficult to isolate the knot fungus from infected tissues even though it was known to be present. Occasionally when plugs of infected tissue were placed on artificial media, the host tissue continued to become hypertrophied and the fungus produced first a conidial stroma bearing a few conidia on the plug of tissue and later immature perithecia. On no occasion, however, were these cultures induced to complete their development to the production of mature perithecia containing mature ascospores of *D. morbosum*.

Chlamydosporous Stage

In addition to a perithecial stage, *D. morbosum*, and a conidial stage, *Hormodendrum* sp., the black knot pathogen was proved in a previous article (*11*) to produce a chlamydosporous stage (Plate II, 10) under certain conditions. The interesting discovery was made that *D. morbosum* produced chlamydospores only when grown in close proximity to certain other organisms, or when chemicals were introduced into the culture medium. Chlamydospore formation was also induced on twigs of *Prunus domestica* when *Hormodendrum* conidia germinated in intimate association with antagonistic bacteria originally isolated from the same host.

CULTURAL STUDIES

Since the conidial or *Hormodendrum* stage is the actively parasitic one throughout most of the growing season its growth reaction in pure culture to various temperatures and to different culture media was studied.

TABLE 1.—SOURCES OF ISOLATION AND DISTINGUISHING FEATURES ON CERTAIN MEDIA OF FOUR CONIDIAL (HORMODENDRUM) STRAINS OF *D. morbosum*

Strain	Source of isolation	Distinguishing features		
		On potato $2\frac{1}{2}\%$ dextrose agar	On Czapek's synthetic agar	On sterile, sour cherry fruits
X	(1) Monoascospores from knots on *Prunus domestica* and *P. pennsylvanica*. (2) Incipient knot swellings. (3) Conidia on knots produced both naturally and artificially.	Margin of colony indented and very irregular. Narrow white border. Zonation. Under side of colony dark. Upper surface of colony, dark greenish olive.*	As on p.d.a.	Very dark green, sparse mycelium, short conidiophores.
Y	(1) Monoascospores as in X. (2) Incipient knot swellings. (3) Conidia on knots outdoors.	Margin of colony regular. Wide white border. Under side of colony light yellow, sometimes pigmenting the medium. Upper surface of colony, dull citrine.*	As on p.d.a.	
Z	(1) Monoascospores as in X. (2) Incipient knot swellings. (3) Conidia on knots produced both naturally and artificially.	More aerial mycelium than X or Y. Under side of colony frequently pinkish, sometimes pigmenting medium. Usually producing sectors. Zonation. Upper surface of colony, deep olive.*	Less aerial mycelium than on p.d.a.	
C	(1) Monoascospores as in X. (2) Incipient knot swellings on *P. domestica* and *P. cerasus*. (3) Condia on knots outdoors.	Abundance of aerial mycelium. Long conidiophores. Conidia less abundant than in X, Y or Z. Upper surface of colony, deep olive gray.*	Very dark in colour with with white border. Aerial mycelium not as abundant as on p.d.a.	Light olive gray,* abundance of aerial mycelium, Conidiophores long and sparse.

*According to Colour Standards and Colour Nomenclature by Ridgway.

Comparisons of the growth characteristics of the four strains of *Hormodendrum* mentioned previously are given in Table 1. Colonies from which descriptions were made were 7 days old and were growing in Petri dishes containing 10 cc. media at 22–23° C.

It will be observed from an examination of this table that these four strains were distinguished both by their colour and growth reactions on certain media (Plates III and IV). On certain other media, *e.g.*, plum decoction and Leonian's malt the four strains also proved to be distinguishable but not as readily as on the media tabulated.

Cultures were also grown at the following temperatures:—5–7° C., 8–10° C., 10–12° C., 31–32° C.

The relative growth rates of four conidial strains namely X, Y, Z and C at three different temperatures on potato 2.5% dextrose agar are shown in Figure 2 where the maximum diameter of the colony growing in 10 cc. media on a Petri dish in each case has been plotted against the length of the growth period. Growth occurred throughout the unusually wide temperature range 3–33° C. It will be observed in the graph that 7–9° C. was evidently below the optimum, while the highest temperature 31–32° C. was above it. At 22–24° C. growth was rapid and steadily maintained for all strains. Strain Z grew more rapidly at all temperatures and had a somewhat wider range than any of the other three strains. A marked parallelism in relative growth rates of the four strains was observed at different temperatures. The rapidity of growth was in nearly all cases in the order X, Y, Z

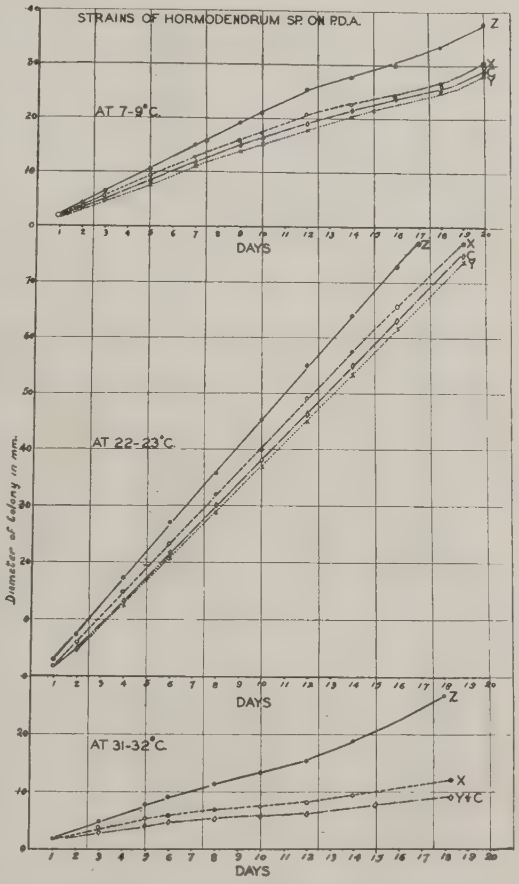

FIGURE 2. Graph illustrating the relative growth rates of 4 conidial strains (*Hormodendrum* sp.) of *D. morbosum* on potato 2½ per cent dextrose agar at 3 different constant temperatures.

and C respectively, though in several cases (*e.g.* 31–32° C.) the growth rate of Y and C fell along practically the same line.

It will be observed from the above that the strains X, Y, Z and C can in nearly all cases be distinguished by their relative growth rates at the same and at different temperatures. Another outstanding feature of the above experiments was the ability of all four strains of *Hormodendrum* to grow at relatively low temperatures. It seems probable from these experiments that *D. morbosum* is active at considerably lower temperatures than are favourable for the growth of its host.

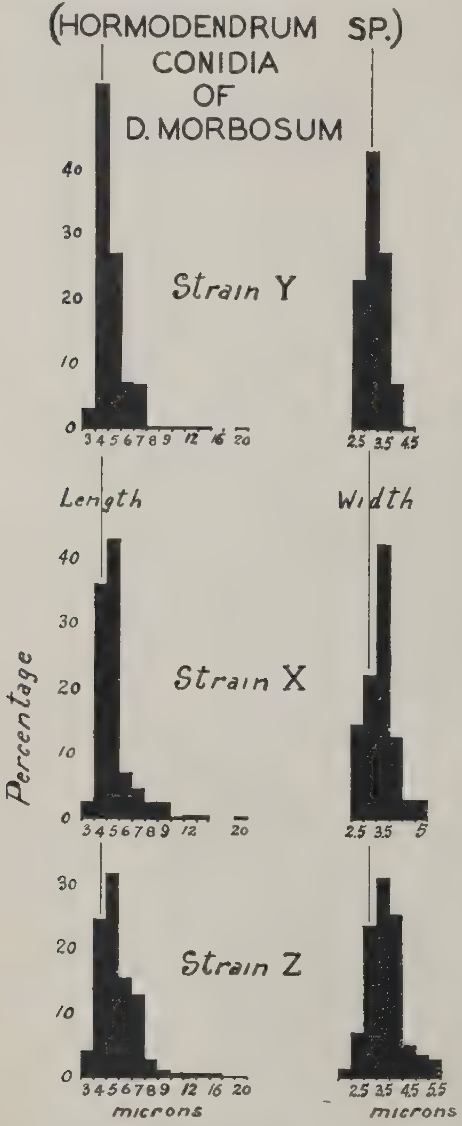

FIGURE 3—Graph illustrating distribution curves of sizes of spores of 3 conidial (*Hormodendrum*) strains of *D. morbosum*.

All four of the above-mentioned conidial strains have been cultured and sub-cultured many times. Generally speaking, no changes were observed in them even after sub-culturing them for several years. Strain Z was observed to produce sectors consistently and frequently, while sectors were produced by other strains relatively infrequently. Curiously enough, the sectors produced by strain Z always appeared to be identical. After sub-culturing numerous of these "sector" colonies for a period of 6 months or more, occasionally one reverted to what appeared to be the parent strain, but, generally speaking, the sector apparently remained as it was when first transferred.

Conidia of all strains were stained with Haidenhain's iron-alum haematoxylin and were found to vary from a single to multinucleate condition. The majority of the conidia of strain Z, however, appeared to be multinucleate while on the other hand the majority of conidia belonging to strains Y, X and C appeared to uninucleate. It therefore seemed possible that some relation existed between the nuclear condition of strain Z and its habit of sectoring.

A relatively small proportion of conidia of all strains germinated on potato dextrose agar at room temperature in 12 to 24 hours (Plate II, 11) while in distilled water very little germination occurred. After several days in distilled water some free budding of conidia occurred and occasionally a few conidia produced germ tubes and a small amount of mycelium.

In an attempt to distinguish strains by their respective spore sizes, measurements were made of 256 conidia of the three *Hormodendrum* strains isolated most frequently, namely X, Y and Z and the percentages of conidia occupying each size unit was calculated. These comparative percentages are illustrated graphically in Figure 3. It will be observed from an examination of this graph that the modes for the length and width of conidia of strain Y lie at 4 and 3 microns respectively, while similar modes for conidia of each of the other two strains X and Z lie at 5 and 3.5 microns respectively. While the modes for both length and width of conidia of strains X and Z lie at the same points strain Z possesses a larger percentage of both longer and wider conidia than strain X which approximates the former most closely in size.

Thus from the above we conclude that the conidia of strain Y are smaller both in length and width than the conidia of either strain X or strain Z. On the other hand strains X and Z cannot be as easily distinguished by the size of their conidia though there is more variability in strain Z than in strain X.

DISCUSSION

The present investigation has yielded new and additional information regarding the symptomatology of the black knot disease and the life history of the causal organism. Many of the observations recorded by the earlier investigators of black knot, particularly those relating to symptomatology though never confirmed have been accepted unquestioned, and as a consequence are found even in the latest text books.

The discovery that more than 95% of primary black knot infection occurs on current season twigs alters the conception formerly held, namely, that branches of all ages are infected. It also suggests a high type of parasitism on the part of the pathogen. Nearly all knots resulting from spring infection were detected in the fall of the same year as small swellings on the twigs. Some of these incipient knots produced an abundance of conidia during the fall of the year of infection and perithecia during the following winter, thereby completing the life cycle of the pathogen on the host within a year. Thus, contrary to prevailing opinion, *D. morbosum* in so far as it is capable of completing its life cycle within a year on the host does not differ from *Venturia inaequalis* and many other Ascomycetes parasitic on fruit trees.

At first sight it seems surprising that the knowledge of the life history of a pathogen as widespread and as destructive as *D. morbosum* has remained so long incomplete and that no contributions in this connection have appeared in the last twenty years. However, there are extenuating circumstances to account for the hindrance to the solution of the problem.

When ordinary cultural technique is employed only rarely do ascospores continue development beyond the formation of germ tubes. Furthermore, numerous other fungi are always found associated with black knots, except those in incipient stages. Finally, there is considerable difficulty in obtaining successful artificial infection. The conidial stage, though it commonly develops on young knots, has never previously been identified as a species of *Hormodendrum* because it has never been critically studied in culture. As it appears on the host the conidial stage cannot be definitely assigned to any genus because its true characteristics rarely, if ever, appear as it is exposed to wind and rain both of which tend to remove the majority of conidia as soon as they mature and consequently the conidiophores never become dendroid, nor do the conidia develop in long chains as they invariably do in culture.

During the present investigations the history of two ascospores of *D. morbosum* has been followed by means of photomicrographs from the initial stages of germination to the final formation of *Hormodendrum* conidia 68 to 74 hours later. The chlamydosporous stage of *D. morbosum* has proved interesting not only because the chlamydospores were found capable of remaining viable throughout the winter but also because they were produced in culture only in the presence of certain other organisms or of chemicals. The isolation of four morphologically distinct conidial strains of *Hormodendrum* sp. from monoascospores of *D. morbosum* indicates a resemblance in this respect to numerous other fungi in which more than one morphologically distinct conidial strain has been demonstrated. These strains suggest the occurrence of physiological forms.

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Résumé

Recherches sur le "Black Knot" ou nœud noir du prunier et du cerisier, III. Symptomatologie, cycle évolutif et culture du *Dibotryon morbosum* (Sch.) T. & S. L. W. Koch, Laboratoire fédéral de pathologie végétale, St. Catharines, Ont.

Contrairement à l'opinion que l'on entretenait jusqu'ici, il a été démontré que le *Dibotryon morbosum* peut compléter son cycle évolutif dans le cours d'une année et que la majorité des infections primaires prennent naissance sur les rameaux de la saison. Des observations minutieuses et des photomicrographies d'ascospores à germination unique, dont la majorité produisent des tubes germinatifs et ne continuent pas à se développer, ont démontré pour la première fois que la phase imparfaite du *D. morbosum* est de l'espèce *Hormodendrum*. Une phase de Chlamydospore s'est également développée en certaines occasions. La phase conidiale sur l'hôte diffère de celle de la culture. Quatre espèces conidiales distinctes l'une de l'autre au point de vue morphologique (*Hormodendrum*) ont été isolées des monoascospores du *D. morbosum*.

STUDIES ON THE PHYSIOLOGY OF THE OAT SMUTS[1]

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Ustilago avenae (Pers.) Jens. and *U. levis* (K. and S.) Magn., the two fungi causing smut of oats, occur in every important oat growing region of the world, and are frequently responsible for serious reductions in yield. These two species may be distinguished from each other by the appearance of their spores, those of *U. avenae* being echinulate, whereas those of *U. levis* are smooth. It is also usually considered that they produce different effects on their host-plants. Thus, *U. avenae* may cause rather complete destruction of the floral parts of its host, giving rise to the disease known as "loose smut." The sori of *U. levis*, however, may be entirely concealed by the glumes, a condition known as "covered smut."

Under field conditions considerable difficulty may be experienced in distinguishing these two species from each other by the manner in which they attack oat panicles. Frequently it is found that heads of the "covered" type contain echinulate spores, and sometimes heads of the "loose" type may contain smooth spores. Such observations appear to throw some doubt upon the value of the spore-wall markings, and the relationship between them and the appearance of the diseased panicles, as reliable specific characters. To obtain further information on these points experiments with pure cultures of the two oat smuts were begun at this laboratory in 1929. A preliminary report (*6*) on this investigation was published in 1930.

EXPERIMENTAL METHODS

Single chlamydospores of *U. avenae* and *U. levis* collected on Longfellow oats were germinated in hanging drops of Difco potato-dextrose-agar, and their sporidia were removed and cultured separately, according to the method described by Hanna (*4*). The temperature of germination was kept between 18° and 20° C. The position on the promycelium occupied by each sporidium was noted, and the four monosporidial cultures obtained from each promycelium were designated accordingly. When a monosporidial culture in a hanging drop had reached a diameter of about 3 mm., it was transferred to a test tube of potato-dextrose-agar. Using this method, the four sporidia from each of 10 chlamydospores of *U. avenae* and 13 chlamydospores of *U. levis* were removed and cultured separately.

Dehulled seeds of Longfellow and Abundance oats were treated with formalin and germinated on moist filter-paper. Some lots of these seedlings were inoculated with single monosporidial cultures of *U. avenae*, others with single monosporidial cultures of *U. levis*, and others with pairs of cultures of *U. avenae* or of *U. levis*. Other inoculations were made by mating a single monosporidial culture of *U. avenae* with one of *U. levis*. The seedlings were inoculated when their coleoptiles were about 5 mm. or less in length. The method of inoculation was to rub some of the sporidia

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on the surface of the coleoptile, after which the seedling was kept on moist filter-paper or sand until the first leaf had formed. It was then transplanted, and grown to maturity either in the greenhouse or in the field. The panicles of the mature plants were examined for smut infection, and notes were taken on the appearance of the smutted heads and on the kind of spores found in them.

Pathogenicity of Monosporidial Cultures Used Singly and in Pairs

Plants grown from seedlings inoculated with a single monosporidial culture of either species showed no sign of infection and failed to produce smutted heads. This conclusion is based on the results of inoculations made with 114 single monosporidial cultures.

Forty-six cultures of *U. avenae* and 58 cultures of *U. levis* were used in making the inoculations with paired monosporidial cultures. The *U. avenae* cultures originated from complete sets of four sporidia isolated from each of 10 chlamydospores, and sets of three sporidia isolated from each of 2 chlamydospores. The *U. levis* cultures originated similarly from all four sporidia from each of 13 chlamydospores, and sets of three sporidia from each of 2 chlamydospores. Plants grown from seedlings inoculated with certain pairs of monosporidial cultures of *U. avenae*, or of *U. levis*, or with certain monosporidial cultures of *U. avenae* paired with certain others of *U. levis* produced smutted heads, whereas plants grown from seedlings inoculated with other such pairs of cultures did not. Hence, it was concluded that the monosporidial cultures belonged to two different sexual groups, and that smutted heads were produced only by those plants which had been inoculated with two monosporidial cultures of opposite sex. On this basis, each set of monosporidial cultures originating from a single chlamydospore could be divided into two sexual groups, designated as (+) and (−). Furthermore, as monosporidial cultures originating from different chlamydospores, whether of *U. avenae* or of *U. levis*, mated with one another in the same ratio as did those from a single chlamydospore, it was concluded that the same two sexual groups were present in both species.

Distribution of Sporidia of Diverse Sex on the Promycelium

Other investigators (*2*), (*5*), (*7*), have shown that in the genus *Ustilago* the distribution of sporidia of different sexes on the promycelium is determined at the time of reduction-division. If reduction occurs at the first division of the fusion-nucleus the arrangement of the nuclei and sporidia will be as follows: (++−−), or (−−++); if at the second division: (+−+−), (−+−+), (+−−+), or (−++−). In the present investigation the distribution of the sporidia of different sexes on the promycelium was determined by inoculation experiments for 10 chlamydospores of *U. avenae* and 13 of *U. levis*. In addition, sets of three sporidia were removed from the promycelia of 2 other spores of each species and, after the sex to which these sporidia belonged had been determined, it was possible to establish by inference the sex of all four sporidia from each spore. In 4 spores of *U. avenae* and 3 of *U. levis* it was concluded that reduction of the factors for sex occurred at the first division of the fusion-nucleus, and in 8 spores of *U. avenae* and in 12 of *U. levis* at the second

division. Thus, in 7 of the spores reduction occurred at the first nuclear division, and in 20 at the second division. It is possible that different results might have been obtained had these spores been germinated on other media or at a higher or lower temperature. Bauch's (*1*) experiments with *U. longissima* indicate that relatively high temperatures favour reduction at the first division; and Dickinson (*3*) has shown that the time of segregation may be affected by the hydrogen-ion concentration of the medium, the temperature of germination, and the total concentration and relative proportions of carbohydrate and nitrogen in the medium.

The Inheritance of Spore Characters

Plants inoculated with two monosporidial cultures of opposite sex of *U. avenae* produced echinulate chlamydospores, and those inoculated with a pair of such cultures of *U. levis* produced smooth chlamydospores. The appearance of the spore-wall, therefore, may be regarded as a reliable character for differentiating the two species.

When plants were inoculated with a monosporidial culture of *U. avenae* and one of opposite sex of *U. levis*, the hybrid-chlamydospores formed in the smutted heads were echinulate, and identical in appearance with those of the *U. avenae* parent. There is every reason to believe that this cross occurs frequently under natural conditions, in which case the hybrid-chlamydospores would be mistaken for chlamydospores of *U. avenae.* Holton (*7*) obtained the same results in his inoculation experiments with pure cultures of the oat smuts.

Considerable difficulty was experienced in culturing the single sporidia from germinated hybrid-chlamydospores resulting from crosses between *U. avenae* and *U. levis*. However, a few such cultures were obtained, and 10 of them were used in inoculation-experiments to determine the manner in which sex and spore-wall character are inherited. Nine of the pairings made between certain of these cultures gave rise to echinulate chlamydospores, and 7 gave rise to smooth chlamydospores. Back-crosses made between certain of the hybrid-cultures and cultures of *U. levis* yielded 4 combinations which produced echinulate chlamydospores and 7 which produced smooth chlamydospores. Similar back-crosses were made with *U. avenae* and, of the 6 fertile combinations obtained, all produced echinulate chlamydospores. Summarizing the results of all the matings, it is concluded that 4 of the 10 hybrid cultures were (+) in reaction and carried the factor for smooth spore-wall, whereas the remaining 6 were (−) in reaction, 4 of them carrying the factor for echinulate spore-wall and 2 the factor for smooth spore-wall. These data show also that the factor for echinulate spore-wall is dominant over that for smooth spore-wall.

The Inheritance of Type of Smutted Head

In a preliminary report of this investigation (*6*) it was stated that plants inoculated with two cultures of opposite sex of *U. levis* produced heads of the "covered" type, whereas plants inoculated with similar cultures of *U. avenae*, or with a culture of *U. avenae* and one of opposite sex of *U. levis* produced heads of the "loose" type. This conclusion was based on the results of inoculations made in the greenhouse. Since then many

TABLE 1.—THE RELATION BETWEEN THE KIND OF CHLAMYDOSPORES PRODUCED ON OATS INOCULATED WITH PAIRS OF MONOSPORIDIAL CULTURES AND THE APPEARANCE OF THE INFECTED HEADS

Chlamydospores	Kind of chlamydo-spores	Total number of heads	Number of "covered" heads	Number of "loose" heads	Number of powdery heads	Percentage of "covered" heads	Percent-age of "loose" heads	Percentage of powdery heads
Grown in the Greenhouse								
Ustilago avenae	Echinulate	405	146	259	346	36.0	64.0	85.4
U. levis	Smooth	305	258	47	9	84.6	15.4	3.0
F_1 (*U. avenae* × *U. levis*)	Echinulate	284	150	134	224	52.8	47.2	78.9
F_2 (*U. avenae* × *U. levis*)	Smooth	77	42	35	1	54.5	45.5	1.3
F_2 (*U. avenae* × *U. levis*)	Echinulate	49	15	34	32	30.6	69.4	65.3
F_1 (*U. avenae* × *U. levis*) × *U. levis*	Smooth	47	22	25	4	46.8	53.2	8.5
F_1 (*U. avenae* × *U. levis*) × *U. levis*	Echinulate	33	17	16	11	51.5	48.5	33.3
Grown in the Field								
U. avenae	Echinulate	39	22	17	39	56.4	43.6	100.0
U. levis	Smooth	66	66	0	10	100.0	0	15.2
F_1 (*U. avenae* × *U. levis*)	Echinulate	140	101	39	140	72.1	27.9	100.0

more inoculated plants have been grown both in the greenhouse and in the field, and somewhat different results have been obtained. It is now apparent that the classification of heads into "loose" and "covered" types is much more difficult than the classification of spores on the basis of spore-wall character. Furthermore, the character of the spore-wall cannot always be predicted from the appearance of the heads in which the spores are borne. The nature of the spore-wall seems to be determined solely by the genetic constitution of the parasite. In the experiments made thus far, it has proved to be independent of the variety of the host-plant and the conditions of growth. The appearance of the infected head, however, probably results from the interaction of parasite, host, and the conditions under which the latter is grown. Under field conditions, as is shown in Table 1, plants inoculated with cultures bearing the factor for smooth spore produce a much higher proportion of heads of the "covered" type than do those inoculated with cultures bearing the factor for echinulate spore. Yet, in plants inoculated with similar cultures and grown in the greenhouse, the differences are much less marked. Apparently, therefore, the inherent capacity of the parasite to affect its host in a particular way may be modified by environmental conditions. Some extreme variations in the appearance of infected heads are shown in Figures 1 and 2.

The results of these experiments are not in complete agreement with those of Holton (7) who states: "Artificial inoculations with crosses between monosporidial lines of *Ustilago avenae* produced both the loose and covered smuts. *Ustilago levis* produced only the covered smut. Crosses between *U. avenae* and *U. levis*, produced loose, covered, and intergrading types of smut."

The nature of the spore-mass, whether "powdery" or otherwise, determines the extent to which, under field-conditions, the spores will be dispersed by the wind. Notes were taken, therefore, on the consistency of the spore-masses in the infected heads. If the chlamydospores fell out readily when the heads were tapped against a sheet of white paper, the heads were classified as "powdery." This classification of the heads is included in Table 1. If "looseness" is considered to result not in the more or less complete destruction of the glumes, but in the production of sori containing powdery masses of spores, then it is evident from the data presented that cultures bearing the factor for echinulate spore usually produce a much higher proportion of heads of the "loose" type than do those bearing the factor for smooth spore.

Spore Germination and Sporidial Growth

Chlamydospores of different collections of *U. avenae, U. levis*, and the hybrid (*U. avenae* × *U. levis*) have been tested for germination and, although differences were found in the germinability of individual collections of spores, it was not apparent that spores of the parent species differed from one another or from the hybrid in their ability to germinate. The percentage germination of different kinds of chlamydospores is given in Table 2.

The pathogenicity of F_1 hybrid-chlamydospores was compared with that of the two parent species by dusting oat-seed with the three kinds

FIGURE 1. Extremes of variation in the appearance of smutted oat panicles produced on plants inoculated with pairs of monosporidial cultures. Left, two heads of "loose" smut *(Ustilago avenae)*; right, two heads of the "loose" type produced by *U. levis*.

FIGURE 2. Extremes of variation in the appearance of smutted oat panicles produced on plants inoculated with pairs of monosporidial cultures. Left, two heads of the "covered" type produced by *Ustilago avenae;* right, two heads of "covered" smut *(U. levis)*.

FIGURE 3. Photomicrograph of an F_1 hybrid *(Ustilago avenae × U. levis)* chlamydospore which has germinated but failed to develop further. × 800.

TABLE 2.—THE GERMINATION OF INDIVIDUAL OAT SMUT CHLAMYDOSPORES AND THE GROWTH OF THEIR SPORIDIA IN HANGING DROPS OF POTATO-DEXTROSE-AGAR

Chlamydospores	Kind of chlamydospores	Number placed to germinate	Number germinating	Percentage germination	Number producing colonies of sporidia	Percentage producing colonies of sporidia
Ustilago avenae	Echinulate	236	144	61.0	52	36.1
U. levis	Smooth	1033	642	62.1	410	63.9
F^1 (*U. avenae* × *U. levis*)	Echinulate	542	337	62.2	31	9.2
F^2 (*U. avenae* × *U. levis*)	Smooth	60	42	70.0	31	73.8
F^2 (*U. avenae* × *U. levis*)	Echinulate	178	90	50.6	62	68.9
F^1 (*U. avenae* × *U. levis*) × *U. levis*	Smooth	208	130	62.5	76	58.5
F^1 (*U. avenae* × *U. levis*) × *U. levis*	Echinulate	140	91	65.0	67	73.6

of spores and sowing it in pots in the greenhouse. Sixty-nine per cent of the plants grown from seed inoculated with the F_1 spores were smutted, as compared with 94 and 90% respectively for the plants grown from seed inoculated with spores of the *U. avenae* and *U. levis* parents. It may be concluded, therefore, that considerable numbers of sporidia or hyphae from the F_1 hybrid-chlamydospores are capable of combining sexually and infecting young oat-seedlings.

In studying the germination of F_1 hybrid-chlamydospores it was noticed that, although the spores germinated normally, many of their sporidia were unable to continue growth on artificial media. The sporidia of these spores either failed to grow, or elongated slightly into short hyphae which subsequently died. This condition is illustrated in Figure 3. The relative ability of different kinds of chlamydospores to form colonies of sporidia or hyphae when germinated separately in hanging drops of potato-dextrose-agar is shown in Table 2. Relatively few of the F_1 hybrid-chlamydospores succeeded in producing colonies as compared with those of *U. avenae* or *U. levis*. It is also significant that the F_2 (*U. avenae* × *U. levis*), and the F_1 (*U. avenae* × *U. levis*) × *U. levis* chlamydospores formed colonies as readily as did those of *U. avenae* or *U. levis*. This would seem to indicate that the factor affecting the growth of the sporidia of F_1 hybrid-chlamydospores was not operating in the F_2 hybrid-chlamydospores and in those produced by back-crossing the F_1 hybrid with the *U. levis* parent.

In other experiments individual sporidia were removed from germinating chlamydospores of *U. levis* and of the F_1 hybrid (*U. avenae* × *U. levis*) and were placed in hanging drops of nutrient-medium. The subsequent development of these sporidia was then observed. Some of them multiplied and produced colonies, but others failed to grow. The results of the experiments have been summarized in Table 3. In this table the two parent monosporidial cultures of each collection of chlamydospores are given, and the sporidia isolated from different kinds of chlamydospores have been considered separately. The subscript attached to the number designating each parent monosporidial culture refers to the position of the sporidium on the promycelium, and the number or letter preceding it to the chlamydospore from which the sporidium came. Thus, $2BO_1$,

$2BO_2$, $2BO_3$, and $2BO_4$, refer to cultures of the 1st, 2nd, 3rd, and 4th sporidia of spore 2BO, the sporidia being numbered from the apex of the promycelium to the spore.

TABLE 3.—THE GROWTH ON NUTRIENT-AGAR OF INDIVIDUAL SPORIDIA REMOVED FROM GERMINATING CHLAMYDOSPORES OF *Ustilago levis* AND OF THE F_1 HYBRID *(U. avenae × U. levis)*

Parent monosporidial cultures of the chlamydospores	No. of sporidia isolated	No. of sporidia which grew	Per cent of sporidia which grew
(U. levis × U. levis)			
$2BO_1 \times 2BO_2$	54	24	44
$2BO_1 \times 2BO_4$	11	11	100
$2BO_2 \times 2BO_3$	64	26	41
$2BO_3 \times 2BO_4$	22	22	100
$2B3_3 \times 2B3_4$	51	51	100
$2B3_1 \times 2B3_4$	22	22	100
$2BJ_1 \times 2BJ_2$	12	12	100
$2BJ_1 \times 2BJ_3$	21	19	90
Total	257	187	73
(U. avenae × U. levis)			
$D1_3 \times G2_1$	100	0	0
$D1_1 \times G2_2$	36	0	0
$D1_2 \times G2_2$	76	4	5
$D1_1 \times G2_4$	48	1	2
$D1_2 \times G2_4$	100	10	10
Total	360	15	4

From the data given in Table 3 it would appear that the sporidia of certain collections of chlamydospores of *U. levis* are more difficult to culture than those of others, and that between collections of the F_1 hybrid-chlamydospores the variability in this respect is still more pronounced. Of the 360 individual sporidia isolated from F_1 hybrid-chlamydospores, only 4 per cent continued growth and formed colonies, whereas of 257 sporidia similarly isolated from spores of *U. levis*, 73 per cent formed colonies. Complete sets of four sporidia were removed from numbers of germinating F_1 hybrid-chlamydospores, but in no instance did all four continue growth and produce colonies. The greatest number of monosporidial cultures obtained from a single one of these spores was two, and frequently all four sporidia from a hybrid-spore failed to grow. Yet, no such difficulty was experienced in culturing all four sporidia from individual chlamydospores of *U. avenae* or *U. levis*. Apparently many sporidia of the F_1 hybrid-chlamydospores are unsuited physiologically to propagate themselves on nutrient-media. This peculiarity may be due to inability of the chromosomes of the two parent species to mate completely with one another. Holton (7) experienced the same difficulty in making monosporidial cultures from F_1 hybrid-chlamydospores.

In making monosporidial cultures from a collection of chlamydospores of *U. levis* it was observed that the growth produced by certain sporidia

TABLE 4.—SEGREGATION FOR SPORIDIAL AND HYPHAL TYPES OF GROWTH IN GERMINATING CHLAMYDOSPORES OF *Ustilago levis* PRODUCED BY MATING SPORIDIAL AND HYPHAL TYPES OF MONOSPORIDIAL CULTURES DERIVED FROM A SINGLE CHLAMYDOSPORE.

Chlamydospores from the cross: hyphal colony No. 2 (−) × hyphal colony No. 3 (+)				Chlamydospores from the cross: sporidial colony No. 1 (+) × sporidial colony No. 4 (−)				Chlamydospores from the cross: sporidial colony No. 4 (−) × hyphal colony No. 3 (+)				Chlamydospores from the cross: sporidial colony No. 1 (+) × hyphal colony No. 2 (−)			
1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
H	H	H	H	S	S	S	S	H	S	H	S	H	S	H	S
H	H	H	H	S	S	S	S	S	H	H	S	H	S	H	S
H	H	H	H	S	S	S	=	S	H	H	S	O	O	S	H
H	O	H	O					S	S	H	H	O	O	S	H
O	H	O	H					*	H	=	*	H	O	O	S
H	O	O	H					=	*	H	H	O	O	S	S
H	H	O	O									O	H	O	H
O	H	H	O									S	H	O	O
*	*	H	H									O	S	S	O
O	O	O	O									=	H	S	=
O	O	O	O									O	O	O	O
O	O	O	O									O	O	O	O
O	O	O	O									O	O	O	O
O	O	O	O									O	O	O	O
O	O	O	O												
O	O	O	O												

NOTE: Numbers 1, 2, 3, and 4 indicate position of sporidia on promycelium, numbering from the apex to the base. The following symbols have been used: (H), for hyphal colony. (S), for sporidial colony. (*), for a colony composed of hyphae and sporidia. (=), for sporidia which were not isolated. (O), for sporidia which were isolated and failed to grow.

in hanging-drop cultures of potato-dextrose-agar consisted entirely of hyphae, whereas that produced by others consisted of sporidia. These two types of growth could be distinguished under the microscope one or two days after the sporidia had been removed from the promycelium, but later when the cultures had been transferred to test-tubes, the differences between them disappeared and all tended to produce hyphae. Cultures from one of these chlamydospores were used to inoculate oat-seedlings. In making the incoulations sporidia of opposite sex were paired together so that the following combinations of cultural types were secured: (sporidial × sporidial,) (hyphal × hyphal), and (sporidial × hyphal). Chlamydospores resulting from these crosses were then germinated singly and their sporidia were removed and cultured separately in hanging drops of potato-dextrose-agar. The types of colonies produced by sporidia from the three kinds of chlamydospores are listed in Table 4. It will be seen that three of the chlamydospores yielded sporidia producing intermediate types of colonies composed of both sporidia and hyphae. With these exceptions, however, the chlamydospores resulting from the crosses (sporidial × sporidial) and (hyphal × hyphal) produced on germination only sporidial and hyphal types of colonies respectively, while chlamydospores resulting from the cross (sporidial × hyphal) produced the two colony-types in the ratio of 2 sporidial to 2 hyphal on each promycelium. The distribution of the two kinds of sporidia on the promycelium indicates that segregation for cultural type may take place at the first or second division of the chlamydospore-nucleus. Dickinson (*3*) and Holton (*7*), working with *U. levis*, and with *U. avenae* and *U. levis* respectively, have also found that segregation of certain cultural characters may occur at the first or second nuclear division. It is of interest that all four sporidia from certain promycelia failed to produce colonies, and that of other sets of four sporidia only two survived. Even more important is the fact that in none of the groups of four did only a single sporidium grow. This suggests that the capacity of the sporidia to reproduce on artificial media is a heritable character, independent of sex and cultural type.

SUMMARY

1. The sporidia were removed from the promycelia of individual germinating chlamydospores of *Ustilago avenae* and *U. levis*, and cultured separately on nutrient-agar. Oat-seedlings inoculated with these cultures were grown to maturity.

2. Plants inoculated with single monosporidial cultures of *U. avenae* or *U. levis* failed to produce smutted heads.

3. Plants inoculated with two monosporidial cultures of opposite sex of *U. avenae* or *U. levis*, or with a culture of *U. avenae* and one of opposite sex of *U. levis* produced smutted heads.

4. Heads infected with *U. avenae* or with the hybrid (*U. avenae* × *U. levis*) contained echinulate chlamydospores; those infected with *U. levis* contained smooth chlamydospores. Proof of the dominance of the factor for echinulate spore over that for smooth spore was obtained by back-crossing cultures of the F_1 hybrid (*U. avenae* × *U. levis*) with cultures of *U. avenae* and *U. levis*.

5. The appearance of smutted heads, whether "covered" or "loose," is not always a reliable indication of the kind of chlamydospores which they contain. However, plants inoculated with cultures bearing the factor for echinulate spore produce a higher proportion of heads of the "loose" type than do those inoculated with cultures bearing the factor for smooth spore. There is evidence that the appearance of smutted heads is determined by the conditions under which the plants are grown as well as by the genetic constitution of the smut-fungus attacking them.

6. The sporidia of *U. avenae*, like those of *U. levis*, are of two kinds, (+) and (−); the sporidia of the one species mate without difficulty with sexually opposite sporidia of the other species.

7. Segregation for sex and cultural characters (sporidial and hyphal types of colonies) was found to occur at either the first or the second division of the chlamydospore-nucleus.

8. No significant difference was found in the ability of *U. avenae*, *U. levis*, F_1 (*U. avenae* × *U. levis*), F_2 (*U. avenae* × *U. levis*) and F_1 (*U. avenae* × *U. levis*) × *U. levis* spores to germinate, but sporidia of the F_1 (*U. avenae* × *U. levis*) spores, as compared with those of the others, are much less capable of growth on artificial media.

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Résumé

Etudes sur la physiologie du charbon de l'avoine. W. Popp et W. F. Hanna, Laboratoire fédéral de recherches sur la rouille, Winnipeg, Man.

Les sporidies ont été enlevées des promycelia de chlamydospores de *Ustilago avenae* et *U. levis* en germination, et cultivées séparément sur agar nutritif. Des plantules d'avoine inoculées de ces cultures ont été développées jusqu'à maturité. Les plants inoculés d'une seule culture monosporidiale de *U. avenae* ou *U. levis* n'ont pas réussi à produire des épis charbonnés. Les plants inoculés avec deux cultures monosporidiales de sexe opposé de *U. avenae* ou *U. levis* ou avec une culture de *U. avenae* et une du sexe opposé de *U. levis* ont produit des épis charbonnés. Les épis

infectés avec *U. avenae* ou avec l'hybride (*U. avenae* x *U. levis*) contenaient des chlamydospores échinulées; ceux qui étaient inoculés avec *U. levis* contenaient des chlamydospores lisses. On a obtenu une preuve de la dominance du caractère pour les spores échinulées par comparaison à celui des spores lisses, en recroisant les cultures de l'hybride F_1 (*U. avenae* x *U. levis*) avec des cultures de *U. avenae* et *U. levis*. L'apparence des épis charbonnés, "vêtus" ou "non vêtus", n'est pas toujours une indication positive du genre de chlamydospores qu'ils renferment. Quoi qu'il en soit, les plants inoculés avec des cultures portant les caractères pour les spores échinulées produisent une plus forte proportion d'épis du genre "nu" ou "non vêtu" que ne font ceux qui sont inoculés avec des cultures portant le caractère pour les spores lisses. Il y a des preuves que l'apparence d'épis charbonnés est réglée par les conditions dans lesquelles les plants sont cultivés aussi bien que par la constitution génétique du champignon du charbon qui les attaque. Les sporidies de *U. avenae*, de même que celles de *U. levis* sont de deux espèces, (+) et (−); les sporidies d'une espèce s'accouplent sans difficulté avec les sporidies du sexe opposé de l'autre espèce. On a constaté que la ségrégation pour les caractères de sexe et de culture (types de colonies sporidiales et hyphales) se produit à la première ou la deuxième division du nucléus-chlamydospore. Aucune différence significative n'a été constatée dans l'aptitude germinative des spores de *U. avenae*, *U. levis*, F_1 (*U. avenae* x *U. levis*), F_2 (*U. avenae* x *U. levis*) et F_1 (*U. avenae* x *U. levis*) x *U. levis*, mais les sporidies des spores de F_1 (*U. avenae* x *U. levis*) comparées à celles des autres, sont beaucoup moins susceptibles de développement sur milieu artificiel.

ABSTRACTS[1]

INOCULATION STUDIES IN PEACH CANKER. R. S. Willison, Dominion Laboratory of Plant Pathology, St. Catharines, Ontario.

Two distinct species of the genus *Valsa* have been isolated from peach cankers in Ontario and identified as *V. leucostoma* (Pers.) Fr., and *V. cincta* Fr., respectively. The salient differences between these fungi, both in culture and on the host, are briefly discussed.

Monosporous cultures of these two species and of *Sclerotinia americana* (Worm.) Nort. and Ez., were used in series of inoculations, made at intervals of one to four weeks over a period of two years, by means of wounds approximately one-and-one-half by one-half inch, cut in branches previously sterilized by brushing with a 1 to 500 solution of mercuric chloride and washing with distilled water. In order to obtain conditions as natural as possible, the wounds were left exposed to the weather without wrapping of any sort. All wounds were measured periodically to determine the amount and rate of healing or enlargement.

In all series, the history of wounds inoculated with *V. leucostoma* closely approximated that of comparable non-inoculated check wounds.

S. americana produced more or less necrosis during the first two or three weeks after inoculation in those series made during the growing season, but little or none in those made during the winter months. Subsequent to the initial damage, healing progressed steadily except in a few wounds which became naturally contaminated with *Valsa* sp. (in most cases *V. cincta*).

On the other hand, *V. cincta*, in series made during the dormant season and for four or five weeks preceding leaf fall, produced cankers which showed either continuous increase in size or increase alternating with a certain amount of healing. Maximum necrosis was caused by this organism when infection occurred at or about leaf-fall. Inoculations made during the summer months with *V. cincta* were comparatively innocuous.

THE CONTROL OF BROWN HEART IN TURNIPS. D. J. MacLeod and J. L. Howatt, Dominion Laboratory of Plant Pathology, Fredericton, New Brunswick.

Brown heart, described by Hurst (Report of Dominion Botanist for 1929, pp. 216–221), is an important limiting factor in the production of turnips in Eastern Canada. In view of the failure to discover a parasitic origin for this obscure trouble, the authors turned to the hypothesis that a mineral deficiency might be the cause. Field tests revealed that sodium tetraborate (borax) applied directly in the drill at the rate of 10 pounds per acre effected a satisfactory control of this disorder and slightly increased the yield. The efficiency of the borax treatment was not materially affected when applications were made in combination with chemical fertilizer, barnyard manure, sulphur, ground limestone or hydrated lime. At the time of emergence there was a slight marginal chlorosis of the leaves of plants receiving the borax treatment. This condition, however, disappeared after from 10 to 14 days. In a limited number of tests, kelp, barnyard manure and sulphur at the rate of 20 and 40 tons and 1,000 pounds per acre, respectively, also controlled the brown heart, but less efficiently than the borax. Sodium chloride at the rate of 400 pounds per acre increased the disorder.

MAGNESIUM DEFICIENCY IN POTATOES. D. J. MacLeod and J. L. Howatt, Dominion Laboratory of Plant Pathology, Fredericton, New Brunswick.

A condition serving as a limiting factor in potato production in New Brunswick has been under observation for two years. This disorder resembles that described by Chucka (Amer. Pot. Jour. *11* : 29-35. 1934) and Knoblauch and Odland (*Ibid.*

[1] Abstracts of papers presented at the fourth annual meeting of the Canadian Phytopathological Society, Macdonald College, June 25–27, 1934, and published elsewhere.

11 : 35-40. 1934) as being due to magnesium deficiency, for which reason the above title has been tentatively given. Characteristic symptoms are confined to the foliar parts of the plant. In the incipient stages affected plants present a chlorotic appearance. In advanced stages the leaves develop an interveinal necrosis which may expand in size, ultimately involving the entire leaf blade. Leaves so affected, particularly the lower ones, usually drop off the plant. Affected plants usually die prematurely resulting in a material reduction in the yield of tubers. Analyses of soils from fields showing the disorder revealed that there is a marked deficiency in magnesium in these soils. In field trials the disorder was corrected and the yield of tubers increased by supplementing the ordinary fertilizer (4–6–10) with dolomitic lime, keiserite, magnesium sulphate and magnesium carbonate. All the forms of magnesium used in these trials were found to be equally effective when supplying the equivalent of 20 pounds of magnesium oxide per acre.

THE INFLUENCE OF ROOTLET KILLING ON THE GROWTH RESPONSE OF APPLE TREES GROWN IN LIQUID CULTURE. J. C. Roger, Dominion Laboratory of Plant Pathology, Summerland, B.C.

Field observations over a period of years have indicated that rootlet killing is commonly associated with the occurrence of such physiological diseases as drought spot and corky core of apple, gumming of prune, drought spot of apricot and cherry, etc. Laboratory experiments in 1933 on young apple trees grown in liquid culture, which were exposed periodically to varying degrees of rootlet killing, gave the following results. In spite of severe root killing, top growth took place continuously, if not normally or at a common rate, on all trees throughout the experiment. The rate of growth appeared to be influenced by conditions other than rootlet killing, as each change in growth rate took place at a similar time in both treated and untreated trees. Leaf scorch and die back, however, appeared to be related to root injury, as distinct symptoms of these occurred only on the treated trees. Periodic rootlet killing of 100% of the absorbing tissue did not prevent continued growth, and it was evident that absorption took place through this dead tissue.

A similar experiment in 1934 substantiated in general the 1933 results, especially with respect to the ability of trees to absorb moisture for considerable periods of time through dead rootlet tissue.

ON THE MERIT OF TREATING POTATO TUBERS TO REDUCE DISEASE AND LOSS CAUSED BY *RHIZOCTONIA SOLANI* KÜHN. G. B. Sanford, Dominion Laboratory of Plant Pathology, University of Alberta, Edmonton, Alberta.

Data, statistically analyzed, from 11 uniform experiments indicate the effect of treating seed potatoes bearing sclerotia of *Rhizoctonia Solani* on reducing—*(a)* lesions on the stem 32 days after planting, and *(b)* the yield at harvest. The types of soil used included those typical of Eastern Canada, and those typical of the prairie, wooded and organic black soils of Alberta. The sets were treated with effective mercuric chloride solution plus 10% by volume of hydrochloric acid. The disease was significantly transmitted to the stem (32 days after planting) from viable sclerotia on the sets in 8 of the 11 experiments. Significant results from treatment were not obtained in a single case where data were based on the total yield or on the yield of marketable tubers at harvest. As the study was repeated in 1934 complete results will appear later.

STUDIES ON THE OVERWINTERING OF CERTAIN FUNGI PARASITIC AND SAPROPHYTIC ON FRUIT TREES. L. W. Koch, Dominion Laboratory of Plant Pathology, St. Catharines, Ontario.

Dibotryon morbosum (Sch.) T. and S. has been isolated on numerous occasions during the winter from definite chlamydospores on buds and bark of *Prunus domestica*. Pure cultures of *D. morbosum* produced chlamydospores only when grown in close proximity to certain other organisms, or when certain chemicals were introduced into

the Petri dishes. Chlamydospore formation was also produced on twigs of *P. domestica* when conidia of *D. morbosum* germinated in intimate association with a strain of bacteria originally isolated from the same host. *Cladosporium carpophilum* was isolated in several instances from chlamydospores on the bark of *Prunus persica*. This organism also produced chlamydospores both on culture media and on peach twigs. A histological study revealed the presence of two types of twig lesions. *Taphrina deformans* was isolated during the winter from the surface of buds on *P. persica*. Proof is submitted that spores of *T. deformans* can remain viable throughout the winter on twigs of the above-mentioned host. Other fungi, presumably saprophytes, representing the genera *Coniothyrium*, *Cladosporium*, *Hormodendrum*, *Fumago*, and a fungus impossible to identify because it produced chlamydospores only, all developed from chlamydospores occurring on various hosts. The evidence obtained indicates the probability that many more fungi overwinter as chlamydospores than have been reported to do so. Due to their unsatisfactory mycological status the importance of chlamydospores has hitherto been incompletely appreciated. The results of these investigations emphasize the importance of the application of a dormant spray on fruit trees.

THE ANALYSIS OF A COMPLEX MOSAIC OF PRESIDENT POTATO. D. F. Putnam, Department of Botany, University of Toronto, Toronto, Ontario.

The potato variety President, which has for a number of years been grown in Nova Scotia under the name of Never Rot, is often affected by an obscure mosaic disease which differs from all of the standard named potato mosaics. The complex has been analysed and found to be an association of three viruses, two of which are apparently the same as the vein banding and mottle viruses found in the rugose mosaic complex. The third, which produces the typical yellow mottle associated with the disease, has not been reported before. The ring spot virus was not found associated with the complex.

The yellow mottle virus apparently belongs to the X-group. It is not transmitted by the aphid *Myzus persicae*, but is sap transmissible and filterable. It is fairly resistant to ageing and to the action of chemicals, and has a thermal death point of approximately 72° C.

ANNUAL MEETING OF THE CANADIAN PHYTOPATHOLOGICAL SOCIETY

The fourth annual meeting of the Canadian Phytopathological Society was held at Macdonald College, June 26 and 27, 1934. Dr. D. L. Bailey, President of the Society was in the chair during the sessions devoted to the reading of papers or the transaction of the business of the Society. The papers that were read before the Society and that are now appearing in Scientific Agriculture were edited by Dr. J. H. Craigie, of the Editorial Committee. The following Officers were elected for the year 1934-35:

President—Dr. J. H. Craigie, Dominion Rust Research Laboratory, Winnipeg, Man.

Vice-President—Dr. H. R. McLarty, Dominion Laboratory of Plant Pathology, Summerland, B.C.

Secretary-Treasurer—Mr. I. L. Conners, Central Experimental Farm, Ottawa, Ontario. (Elected in 1933).

Councillor—Prof. J. G. Coulson, Macdonald College, Que.

Councillor—Dr. D. L. Bailey, University of Toronto, Toronto, Ontario. (Ex-officio).

BOOK REVIEW

HANDBUCH DER PFLANZENKRANKEITEN (Text book of plant diseases) founded by Paul Sorauer; in six volumes; edited by O. Appel. Sixth revised edition. First volume: non-parasitic and virus diseases. First Part, 123 text illustrations; 602 pages; bound 46 Reichsmark. Second Part, 147 illustrations; 561 pages; bound 44 Reichsmark. 1934. Published by Paul Parey—Berlin, Germany.

When some 54 years ago Sorauer issued the first edition of the now famous handbook, it was a most modest affair. Each new edition grew in size and today we have before us the first volume of the sixth edition in two formidable parts issued by Geheimrat Appel and a series of collaborators, well known in the world of plant pathological science. Each new edition of this well known text book marks something of an epoch, reflecting the vast progress which has been made throughout the world in plant pathology and the control of plant diseases.

The first volume of the sixth edition is of particular interest inasmuch as it has been completely and fundamentally rewritten. Indeed almost every chapter has been prepared by a specialist, which modus in view of the continuous advance in our knowledge of this branch of science, is to be welcomed. It would have been too much of an undertaking to endeavour to do justice to the subject single handed. Dr. Appel, the worthy successor to Sorauer in editing this edition is to be congratulated on the choice of his co-workers and experts.

Volume one of the sixth edition—some 1163 pages print, with 270 illustrations, most of which are refreshingly new and original—is divided into two handy separate parts, devoted to non-parasitic and virus diseases. The text is divided into eleven sections.

By way of making our readers familiar with the scope, it is instructive to glance through the table of contents and to have impressed upon one the extraordinary comprehensiveness of the material provided. Indeed once more is the reader reminded that plant pathology is no longer a phase of mycology nor a science merely devoted to researches on fungus parasites. Sorauer, years ago, emphasized the effect of environment and of predisposition of plants to parasitic fungi, and has provided us with a lasting discourse on non parasitic phenomena, which result definitely in ill health of plants, without any parasitic agent being involved. Thus today plant pathology, more so than ever, is a science devoted to research into the cause and prevention of ill health in plants, and requires the attention of the carefully trained specialist, thoroughly familiar with related sciences in every respect. The scope covered by this science nowadays is reflected by the various sections which are divided into a general and a special part. The first section of the general part has been prepared by Professor K. Braun who reviews the history of plant diseases and noxious insects up to the year 1880. Professor Morstatt follows with section two: General plant diseases with two subsections, I, the nature of diseases, and II, Ecology of disease. The introduction of his two groups makes interesting reading, dealing with the more recent development of plant pathology—pointing out that, after all, the broader conception of the science is but 50 (!) years old, Julius Kühn being the first to publish a textbook on the diseases of plants in 1858. Great credit is given to the development of this study in the U.S.A. where plant pathology no doubt has progressed by leaps and bounds. Morstatt might have taken more cognizance of the great organization of a plant pathological service in the Dominion, but, no doubt, since most of our earlier contributions to this science appeared in foreign journals, the identity was not disclosed—a defect which it is hoped is now offset by two excellent periodicals which will help to identify Canadian workers in future. In passing, it may also be said that Canadian plant disease legislation preceded that of the U.S.A.

The special part by Dr. Merkenschlager commences with a discussion of plant nutrition in its bearing on disease, or, rather, disorders in plants, since it is exclusively devoted to non parasitic causes. He discusses in detail biochemical symptoms such as the pathology of carbon hydrate, of albumin, gaseous metabolisms, etc. The pathology of mineral metabolism: calcium, potash, magnesium, phosphoric acid, nitrogen, etc., is dealt with in detail, followed by sections on excessive nutrition and special non-parasitic phytopathology.

Hiltner then takes up questions of the relation of climate and meteorological factors as causes of disorders, dealing with excess of light, light deficiency, temperature conditions, circulation and lack of water and similar factors.

Wartenberg discusses cold and heat as the cause of death in plants and of injuries to plant life, such as the death of the plant cell through low temperatures, the mechanical effect of frost or heat. The material is carefully selected and conveys to the reader a splendid idea of the significance of these factors as causes of ill health in plants.

K. O. Müller reviews disorders due to internal factors, disturbances in the embryonal period, during growth, or functions generally. Anything of interest during the period of reproduction also receives attention of this author.

Dr. Pfeil discusses unsuitable or unfavourable soil conditions as causes of disorders, physical as well as chemical.

Schlumberger leads a rather comprehensive discussion on wounds due to physical causes, the reaction of plants to wounds generally and with regeneration and correlation following wounds, cell-tissue regeneration, adventition buds, loss and replacement of roots, etc.

The seventh subsection is by Professor Tiegs and is devoted to fume injuries—a chapter always of interest to Canada with its many smelters and problems arising therefrom. This chapter is certainly most comprehensive in describing injuries due to a number of gaseous substances likely to be encountered.

He follows this section with a discussion of the effects on vegetation of waste waters mainly resulting from various industries.

Finally Dr. Köhler contributes a formidable section on virus diseases, some 182 pages in length. In view of the ever increasing importance of virus diseases in crops all over the world, this section is specially valuable. There is the general part, discussing etiology, the pathological morphology, anatomy and physiology. The action of the virus within the host, its transmission, the disease in its relation to external factors, questions of resistance, the virus in vitro—classification and control measures. This chapter is followed by the special part dealing with the viruses of the Solanaceae with fair detail. It is followed by a discussion of virus diseases affecting a host of non-solanaceous plants of which there is an alarming number in almost every climate of the universe. A 41-page index completes the two formidable volumes. Sorauer's Handbuch does not require special commendations and it is generally realized that no library is complete without it. It serves a most useful purpose as a work of reference and has become almost indispensable as such. Dr. Appel, the veteran of German plant pathologists, is to be congratulated in having accomplished a great and difficult task so satisfactorily.

H. T. Güssow.

THE ECONOMIC SITUATION

PREPARED IN THE AGRICULTURAL ECONOMICS BRANCH, DEPARTMENT OF AGRICULTURE, OTTAWA, LARGELY FROM BASIC DATA COLLECTED BY THE DOMINION BUREAU OF STATISTICS

Wholesale prices in Canada registered a slight advance in January, the index rising from 71.2 in December to 71.5. The rise was quite general, each of the sub-indexes being higher than in the previous month with the exception of fibres, textiles, and textile products, which was unchanged. None of the commodity indexes showed a sharp advance, however, though that of animals and their products was most substantial. The index for January, 1935, was nearly a point above that for January, 1934.

Retail Prices—The index number of retail prices showed little change as compared with that for December. Food prices were lower, the index dropping from 69.3 to 68.8. The indexes for fuel and sundries were slightly higher but these advances did not offset the reduction in prices of food.

Physical Volume of Business.—The physical volume of business in December, 1934, showed a decline of a little over four points. Industrial production dropped from 97.0 to 91.0. Mineral production fell from 137.5 to 121.8. Exports of copper were greatly reduced as compared with those in November, in which month, however, they were exceptionally high. The shipments of gold were lower and coal production was slightly below that of the previous month. It should be pointed out that the indexes of physical volume of business, industrial production, and mineral production were all well above those in December, 1933.

In the manufacturing group the index declined from 96.0 in November to 91.8 in December. Manufacture of sugar and salmon exports were lower. Production of flour and oatmeal was not reported. Tobacco releases, boot and shoe production, and textile output were also lower. On the other hand, the index of iron and steel rose from 36.6 to 46.7 and automobile production showed an early advance, though the index was substantially below that for December, 1933. Imports of crude petroleum were lower than in the previous month. The construction index declined from 43.1 to 31.2. Trade employment was substantially better and car loadings were slightly lower than in November. Exports were a little higher and imports somewhat lower.

The movement of grains was very slow. In fact, the rate at which wheat has moved into export markets has been disappointing, and prospects now are that the carryover at the end of the crop year will be larger than anticipated. Live stock marketings were slightly off in December, the index dropping to 67.3, a decline of five and one-half points. Hog marketings were decidedly lower. Cattle shipments were also below those in November, but sheep and calves moved in larger volume. Cold storage holdings were slightly above the level of those at November first.

The December live stock and poultry survey has just been released by the Agricultural Branch of the Dominion Bureau of Statistics. The number of cattle and calves on farms at December, 1934, was 8,484,000, a decrease of 5.2 per cent compared with June 1, 1934, but a somewhat larger number than were on farms at December 1, 1933. The reduction in numbers since June, 1934, has taken place in the Maritime Provinces, Quebec, Ontario, and Manitoba, the decrease in the latter three provinces being relatively smaller than in the Maritimes. In Saskatchewan, Alberta and British Columbia cattle numbers are definitely increasing. Prospects are that the number of cows to be bred during the period of December, 1934, to May, 1935 will be 5.7 per cent lower than in the same period of 1933-34.

The number of milch cows shows an increase of 5.1 per cent during the twelve months ending December 1, 1934. There has been an increase in all provinces except Prince Edward Island and Ontario. Cows for beef are increasing less rapidly than

ANNUAL AND MONTHLY INDEX NUMBERS OF PRICES AND PRODUCTION COMPUTED BY DOMINION BUREAU OF STATISTICS

Year	Wholesale Prices 1926 = 100				Retail prices and cost of services (5)	Production (6) 1926 = 100			
	All commodities (1)	Farm products (2)	Field products (3)	Animal products (4)		Physical volume of business	Industrial production	Agricultural marketings	Cold Storage holdings
1913	64.0	62.6	56.4	77.0	65.4				
1914	65.5	69.2	64.9	79.0	66.0				
1915	70.4	77.7	76.9	79.2	67.3				
1916	84.3	89.7	88.4	92.3	72.5				
1917	114.3	130.0	134.3	119.6	85.6				
1918	127.4	132.9	132.0	134.7	97.4				
1919	134.0	145.5	142.4	152.5	107.2	71.3	65.5	48.1	47.1
1920	155.9	161.6	166.5	149.9	124.2	75.0	69.9	52.6	94.2
1921	110.0	102.8	100.3	108.5	109.2	66.5	60.4	65.2	86.4
1922	97.3	86.7	81.3	99.1	100.0	79.1	76.9	82.6	82.8
1923	98.0	79.8	73.3	95.1	100.0	85.5	83.8	91.4	87.6
1924	99.4	87.0	82.6	97.2	98.0	84.6	82.4	102.5	114.9
1925	102.6	100.4	98.1	105.7	99.3	90.9	89.7	97.2	108.6
1926	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1927	97.7	102.1	99.9	105.7	98.4	106.1	105.6	103.6	110.0
1928	96.4	100.7	92.6	114.3	98.9	117.3	117.8	146.7	112.8
1929	95.6	100.8	93.8	112.5	99.9	125.5	127.4	101.1	109.6
1930	86.6	82.3	70.0	102.9	99.2	109.5	108.0	103.0	128.4
1931	72.2	56.3	43.6	77.6	89.6	93.5	90.4	99.0	125.7
1932	66.7	48.4	41.1	60.7	81.4	78.7	74.0	114.3	120.1
1933	67.1	51.0	45.8	59.6	77.7	79.7	76.8	105.1	115.4
1934	71.6	59.0	53.9	67.6	78.9	94.2	93.6	88.5	114.2
1934									
Jan.	70.6	55.3	47.9	67.8	78.2	86.8	84.5	48.2	108.1
Feb.	72.1	58.0	49.3	72.5	78.7	86.4	84.0	67.1	98.6
Mar.	72.0	56.5	49.5	68.3	79.9	93.1	92.0	63.8	97.0
Apr.	71.1	55.4	48.7	66.6	79.4	92.6	91.4	56.9	94.5
May	71.1	56.9	51.1	66.5	78.5	99.6	99.4	130.6	102.6
June	72.1	59.3	55.5	65.6	78.2	95.8	95.2	97.2	126.1
July	72.0	60.0	57.8	63.7	78.4	95.7	95.6	148.8	116.3
Aug.	72.3	61.6	60.7	63.1	78.7	99.0	99.8	172.8	114.7
Sept.	72.0	61.3	58.9	65.3	79.0	97.1	97.5	127.7	117.7
Oct.	71.4	60.9	55.3	70.4	79.3	95.8	95.3	61.2	128.8
Nov.	71.2	61.2	55.7	70.4	79.4	96.5	97.0	51.2	130.4
Dec.	71.2	61.6	56.0	70.9	79.0	92.4	91.0	36.0	135.7
1935									
Jan.	71.5	61.4	55.7	71.0	78.9				

1. See Prices and Price Indexes 1913-1928, pp. 19-21, 270-289 and 1913-1932, p. 15.

2. Wholesale prices of Canadian products of farm origin only. See Prices and Price Indexes 1913-1932, p. 32, and Monthly Mimeographs 1933 and 1934.

3. Wholesale prices of grains, fruits and vegetables.

4. Wholesale prices of Animals and Animal Products.

5. Including foods, rents, fuel, clothing and sundries, See Prices and price Indexes 1913-1928, pp. 181-185, 290-293. 1926=100.

Prices and Price Indexes 1913-1931, p. 122, and Monthly Mimeographs 1933-1934.

6. Monthly Review of Business Statistics, p. 8, and Monthly Indexes of the Physical volume of business in Canada, supplement to the Monthly Review of Business Statistics, November, 1932.

those kept chiefly for milking. There has been a reduction of 45,000 head of calves compared with December 1, 1933, and 300,700 since June 1, 1934. Moreover, there has been a reduction of 56,000 head of steers, two years and over, since the survey taken at December 1, 1933. The trend of cattle numbers is indicated by the reduction in the number of calves. Shortage of feed in some areas is, however, a contributing factor.

Numbers of hogs have decreased slightly since the first of June, 1934, but there were 61,000 more hogs on farms in December, 1934, than at the same date in 1933. The increase has been accounted for in the Maritime Provinces, Quebec and Manitoba, but elsewhere hog numbers are lower. Farrowings were slightly higher in the period June to December, 1934, and there is a slight tendency to increase farrowings during the next six months. There will likely be an increase during the first half of the year in marketings but in the fall of 1935 it is likely marketings will show no appreciable increase.

The number of sheep and lambs on farms was substantially the same as in the previous year. There have been decreases in the Maritime Provinces and in Ontario, Manitoba, and British Columbia, whereas Quebec, Saskatchewan, and Alberta showed increases, particularly in the two western provinces. The number of ewes to lamb will be slightly less than indicated by the survey taken in December, 1933.

The number of hens and chickens was slightly lower at the end of 1934.

The survey indicates a reduction in cattle population, notably in numbers of calves, an increase of about 5 per cent in the number of milch cows, a small increase in the number of hogs; substantially the same number of sheep and lambs, and a small reduction in the number of hens and chickens on farms.

LA SITUATION ÉCONOMIQUE

PRÉPARÉ PAR LA DIVISION DE L'ÉCONOMIE AGRICOLE, MINISTÈRE DE L'AGRICULTURE, OTTAWA, PRINCIPALEMENT D'APRÈS LES DONNÉES RECUEILLIES PAR LE BUREAU FÉDÉRAL DE LA STATISTIQUE

En janvier les prix du gros au Canada ont enregistré une légère hausse, l'indice passant de 71.2 en décembre à 71.5. Cette hausse a été très générale; à l'exception des fibres, des textiles et des produits textiles qui n'ont pas changé chacun des sous-indices était plus élevé qu'au cours du mois précédent. Elle était légère pour les indices relatifs aux marchandises mais prononcée sur les animaux et leurs produits. L'indice de janvier 1935 était de près d'un point supérieur à celui de janvier 1934.

Prix de détail.—Le chiffre indice des prix de détail n'accusait que peu de changement sur celui de décembre. Les prix des denrées alimentaires étaient plus bas, l'indice tombant de 69.3 à 68.8. Les indices pour le combustible et les produits divers étaient un peu plus élevés mais ces hausses n'ont pas été suffisantes pour couvrir la diminution qui s'est produite dans les prix des denrées alimentaires.

Volume physique des affaires.—En décembre 1934, le volume physique des affaires révélait une diminution d'un peu plus de quatre points. La production industrielle est tombée de 97.0 à 91.0, la production minérale de 137.5 à 121.8. Les exportations de cuivre étaient grandement réduites par comparaison à celles de novembre, mais elles étaient exceptionnellement élevées dans ce mois-là. Les expéditions d'or avaient diminué et la production de charbon était un peu inférieure à celle du mois précédent. Il est à noter que les indices du volume physique des affaires, de la production industrielle et de la production minérale étaient tous bien supérieurs à ceux de décembre 1933.

Dans le groupe manufacturier, l'indice qui était de 96.0 en novembre est tombé à 91.8 en décembre. La fabrication du sucre et les exportations de saumon ont diminué. Il n'a pas été fait rapport de la production de farine et de farine d'avoine. Les ventes de tabac, la production de chaussures, la production textile avaient également diminué. Par contre, l'indice du fer et de l'acier s'est élevé de 36.6 à 46.7, la production des automobiles a enregistré une augmentation quoique l'indice ait été bien inférieur à celui de décembre 1933. Les importations de pétrole brut étaient inférieures à celles du mois précédent. L'indice du bâtiment a baissé de 43.1 à 31.2. Le nombre d'employés dans le commerce avait bien augmenté, mais les chargements de wagons étaient un peu inférieurs à ceux de novembre. Les exportations étaient un peu plus fortes et les importations un peu moins élevées.

Le mouvement des grains a été très lent. En fait, le peu de rapidité avec lequel le blé a été transporté sur les marchés d'exportation a causé un désappointement et il est maintenant à prévoir que le reliquat à la fin de l'année de récolte sera plus considérable qu'on ne le prévoyait. Les ventes de bestiaux ont légèrement diminué en décembre, l'indice tombant à 67.3, soit une baisse de cinq points et demi. Les ventes de porcs ont aussi beaucoup diminué. Les expéditions de bovins étaient également inférieures à celles de novembre, tandis que celles de moutons et de veaux étaient plus considérables. La quantité de produits entreposés au froid était un peu plus forte qu'au 1er novembre.

Les résultats du recensement de décembre sur les bestiaux et les volailles viennent d'être publiés par la Division de l'agriculture du Bureau fédéral de la statistique. En décembre 1934, le nombre de bovins adultes et de veaux sur les fermes était de 8,484,000, soit une diminution de 5.2 pour cent par comparaison au chiffre du 1er juin 1934, mais une légère augmentation sur le chiffre du 1er décembre 1933. La diminution qui s'est produite depuis juin 1934 a eu lieu dans les provincesMaritimes le Québec, l'Ontario et le Manitoba; elle était relativement plus faible dans ces trois dernières provinces que dans les provinces Maritimes. La population bovine est en augmentation dans la Saskatchewan, l'Alberta et la Colombie-Britannique. On

prévoit que le nombre de vaches à faire saillir pendant la période allant de décembre 1934, à mai 1935 sera de 5.7 pour cent moins élevé qu'au cours de la même période de 1933-34.

Le nombre de vaches laitières a augmenté de 5.1 pour cent pendant les douze mois finissant au 1er décembre 1934. Il y a eu augmentation dans toutes les provinces, sauf dans l'Ile du Prince-Edouard et dans l'Ontario. Le nombre de vaches destinées à la boucherie augmente moins rapidement que celles que l'on garde principalement pour la traite. Il y a eu une réduction de 45,000 veaux depuis le 1er décembre 1933 et de 300,700 depuis le 1er juin 1934. En outre, il y a eu une diminution de 56,000 bœufs de deux ans et plus depuis le recensement du 1er décembre 1933. La courbe du nombre des bovins est indiquée par la réduction dans le nombre des veaux, mais le manque d'aliments dans certaines régions est pour quelque chose dans cette situation.

Le nombre de porcs a un peu diminué depuis le 1er juin 1934, mais il y avait, en décembre 1934, 61,000 porcs de plus sur les fermes qu'à la même date en 1933. Cette augmentation a été notée dans les provinces Maritimes, le Québec et le Manitoba; partout ailleurs, le nombre de porcs a diminué. Le nombre de mises-bas a été un peu plus élevé pendant la période qui s'est écoulée entre juin et décembre 1934, et il a une légère tendance à augmenter le nombre des mises-bas pendant les prochains six mois. Il y aura sans doute une augmentation dans les ventes pendant la première moitié de l'année mais il est peu probable que le chiffre des ventes accuse une augmentation sensible dans l'automne de 1935.

Le nombre de moutons et d'agneaux sur les fermes était à peu près le même que l'année précédente. On a enregistré des diminutions dans les Provinces Maritimes ainsi que dans l'Ontario, le Manitoba, et la Colombie-Britannique, tandis que le Québec, la Saskatchewan, et l'Alberta enregistraient une augmentation, spécialement dans les deux provinces de l'Ouest. Le nombre de brebis qui vont mettre bas est un peu moins élevé que l'indiquait le recensement qui a été effectué en décembre 1933.

Le nombre de poules et de poulets était un peu plus faible à la fin de 1934.

Le recensement indique une diminution dans la population des bovins, surtout dans le nombre de veaux, une augmentation d'environ 5 pour cent dans le nombre des vaches laitières, une faible augmentation dans le nombre des porcs, à peu près le même nombre de moutons et d'agneaux, et une faible réduction dans le nombre de poules et de poulets sur les fermes.